RIVM Report 670220001/2001

Quality and safety of products containing Ephedra Herba on the Dutch market

O. A. Lake, C. Slijkhuis, W.F. Maas, M.E.A. van Vliet, D. de Kaste, W. Verdonk-Kleinjan[1]

Gewijzigde herdruk

This investigation was initially within the scope of **MAP SOR** (Project: *Grey Areas; Smart Shop Products*; project number 670210). Presently, it is a project within **MAP Volksgezondheid** (Project number V670220).

RIVM, P.O. Box 1, 3720 BA Bilthoven, telephone: 31 - 30 - 274 91 11; telefax: 31 - 30 - 274 2971
[1] Inspectorate of Health Protection, Commodities and Veterinary Public Health, P.O. Box 2280, 5202 CG 's-Hertogenbosch.

Abstract

We performed analytical studies on dietary supplements and smart products containing Ephedra herba on the Dutch market. Such products are labelled 'from natural, herbal sources' and do not fall under Dutch Medicines Act. Most of the samples tested from 1993 to 1999 contained unacceptably large amounts of ephedrine (EP) alkaloids (the active substances of Ephedra herba) in relation to the safety criteria in the literature. Some samples also contained an effect-enhancing substance (e.g. coffeine), thus potentiating the risks of adverse events. Samples both 'from herbal sources' and 'not from herbal sources' were encountered, and the contents of EP alkaloids varied from product to product, as well as within a product.
This project shows that there is a need to improve the safety and quality of these products in view of public health.

Preface

The National Institute of Public Health and the Environment (RIVM) *Grey Areas Project* covers the field in which the Dutch drug law and regulations meet the laws concerning commodities sold on the Dutch market.

In the Netherlands, certain products in this category are traded with specific labelling and in specific shops, suggesting that these products, and especially dietary supplements and so-called smart products, are of natural, herbal origin.

The products in the *Grey Areas Project* do not fall under the usual regulations for manufacturing, registration, and distribution of drugs because they are not traded with a medical claim.

The principle purpose of the present *Grey Areas Project* is to examine whether smart products, etc., can indeed be considered harmless on the evidence of experimental results and the literature.

The partial project for Ephedra herba products started in 1996 as a *Meerjaren Activiteiten Programma Strategisch Onderzoek RIVM (MAP SOR) Project,* and since 2000, it has been a project of *MAP Public Health*. In this project, the Laboratory for the Quality Control of Medicines (LGO) of the RIVM at Bilthoven analysed samples labelled as containing Ephedra herba. The samples were provided by several inspectorates, and analysis started in 1993. The Inspectorate of Health Protection, Commodities and Veterinary Public Health (KvW) at Den Bosch provided samples from 1997 onwards. The experimental part of the project ended in 1999.

The experimental results, together with a general evaluation and conclusion in relation to public health, are presented in this report.

The investigation was performed under the authority of the KvW, and was financed by the Ministry of Public Health, Welfare and Sports (VWS).

O. A. Lake

Contents

List of abbreviations

CNS	: Central nervous system
EP	: (-)-Ephedrine
FDA	: Food and Drug Administration
GC	: Gas Chromatography
HPLC - DAD	: High-Performance Liquid Chromatography with Diode Array Detection
IGZ	: Dutch Health Care Inspectorate
KvW	: The Inspectorate of Health Protection, Commodities and Veterinary Public Health
LGO	: Laboratory for the Quality Control of Medicines
ME	: (-)-Methylephedrine
MEB	: Medicines Evaluation Board in the Netherlands
MPE	: (+)-Methylpseudoephedrine
NE	: (-)-Norephedrine
NPE	: (+)-Norpseudoephedrine
OTC	: Over the counter
PE	: (+)-Pseudoephedrine
Ph. Eur.	: European Pharmacopoeia
RIVM	: National Institute of Public Health and the Environment
SPE	: Solid Phase Extraction
SYN	: Synephrine
TLC	: Thin-Layer Chromatography
UV	: Ultraviolet
VWS	: Ministry of Public Health, Welfare and Sports

Samenvatting

Op verzoek van de IGZ en KvW heeft het LGO van het RIVM analytisch onderzoek uitgevoerd op Ephedra-producten op de Nederlandse markt.
In totaal 202 monsters van circa 135 producten zijn onderzocht, vanaf 1993 t/m 1999. Deze producten werden verkocht als voedingssupplementen en z.g. smartshopproducten. De beschrijvingen op de etiketten van deze producten en de omgeving waar deze producten werden verkocht (gezondheidswinkels, smart shops etc.), suggereerden dat de producten van natuurlijke, plantaardige oorsprong waren.
Het belangrijkste doel van deze studies was om veiligheid van deze producten te evalueren, aangezien Ephedra herba efedrine alkaloiden bevat. Deze actieve bestanddelen kunnen een duidelijk effect hebben op het cardiovasculaire systeem en het centrale zenuwstelsel, afhankelijk van de toegediende doses. Een tweede doel was, beoordelen in hoeverre de producten werkelijk van plantaardige oorsprong waren d.w.z. in hoeverre bij de productie er werkelijk Ephedra herba was gebruikt, zoals aangegeven op het etiket, en niet een synthetisch bereide stof. Bij de beoordeling van veiligheid en kwaliteit werden criteria uit de literatuur gehanteerd.
Het onderzoek bestond uit drie experimentele studies verricht in respectievelijk de perioden 1993-1996, 1997-1999 en 1999. In die studies ontving het LGO monsters van de diverse overheidsinstituten. Op deze monsters was 'Ephedra herba' vermeld op het etiket of een vergelijkbare naam en/of de vermelding 'van natuurlijke oorsprong'. De analyses bestonden uit bepalingen van identiteit en gehalte van de efedrine (EP) alkaloiden, met behulp van DLC en HPLC-DAD. De monsters van studies 2 en 3 (121 monsters) werden beoordeeld met betrekking tot veiligheid en kwaliteit, die van studie 1: met betrekking tot alleen kwaliteit.
Het grootste deel van de monsters van studies 2 en 3 bevatte onacceptabel hoge gehalten aan EP alkaloïden: in 93 monsters overschreden de gehalten de normen, inclusief een voorstel voor veiligheidscriteria van de FDA. Hiernaast bleken 36 monsters ook een effect potentiërende stof te bevatten (coffeïne) en overschreden in 28 monsters de gehalten de veiligheidslimieten èn bevatten deze monsters ook een effect potentiërende stof, wat een vergroot risico voor de veiligheid betekende. Uit de resultaten bleek tevens dat zowel monsters 'van plantaardige oorsprong' als monsters 'niet van plantaardige oorsprong' aanwezig waren en dat de gehalten aan efedrine alkaloïden fluctueerden, zowel van product tot product als van charges binnen één product.
Uit dit project blijkt dat er behoefte is de veiligheid en kwaliteit van deze producten te verbeteren, ten behoeve van de volksgezondheid.

Summary

The LGO of the RIVM performed analytical studies on products containing Ephedra herba on the Dutch market at the request of the Dutch Health Care Inspectorate (IGZ) and the Inspectorate of Health Protection, Commodities and Veterinary Public Health (KvW). In total, 202 samples from approximately 135 products were examined in the period 1993 to 1999. These products were sold as dietary supplements and so-called smart drugs. The labelling and their sale environment (e.g. health food stores, smart shops) suggested that the products were of natural, herbal origin.

The principle purpose of these studies was to assess the safety risk of these products, considering that Ephedra herba contains ephedrine (EP) alkaloids. These active substances may have pronounced effects on the cardiovascular and central nervous systems, depending on the doses taken. Another purpose was to examine whether the products were indeed of herbal origin, i.e. whether Ephedra herba was indeed used in their production as stated on the label, and not a synthetic substitute. Criteria from the literature were used to assess safety and quality.

The investigations consisted of three separate experimental studies in the periods 1993-1996, 1997-1999, and 1999 in which the LGO received samples from governmental institutes. These samples were labelled 'Ephedra herba' (or a comparable name) and/or 'from natural sources'. The analysis consisted of determining the identity and assay of the EP alkaloids by thin-layer chromatography (TLC) and high-performance liquid chromatography with diode-array detection (HPLC-DAD). The samples for studies 2 and 3 (121 samples) were assessed according to quality and safety criteria; and those for study 1, according to the quality criteria only. Most of the samples of studies 2 and 3 contained unacceptably large amounts of EP alkaloids, and 93 of these samples did not meet the safety criteria, including draft safety requirements of the Food and Drug Administration (FDA). In addition, 36 contained an effect-enhancing substance (coffeine), and 28 of these 36 samples both failed to meet the criteria and contained an effect-enhancing substance, so the risk for adverse events was potentiated. The results also show that samples both 'from herbal sources' and 'not from herbal sources' were encountered and that the total amount of EP alkaloids varied from product to product, as well as within a product.

This project shows that there is a need for improvement in view of public health.

1 Introduction

1.1 Problem statement

In the Netherlands, Ephedra herba products are traded with a specific labelling and in specific shops that suggest that they are of natural, herbal origin. This is true especially of dietary supplements and the so-called smart products. Ephedra herba is a mixture of dried twigs (Figure 1, Appendix 1), from certain plants of the botanical family *Ephedraceae* or comparable [1, 2] and contains several EP alkaloids. Another scientific name for the substance is *Ephedra vulgaris* [2]. Besides 'Ephedra herba', a great variety of popular names used world-wide are given on the labels of these products [3] (Table 1, Appendix 1). Very often, the substance is described as 'Ephedra powder ..% standardised', 'Ephedra extract', 'Ephedra herba ..% standardised', or 'Ma Huang ..% standardised' [4].

In the Netherlands, these products do not fall under the regulations on manufacturing, registration and distribution of medicinal products because they are not traded with a medical claim. In recent years, however, it has become more and more evident that herbal food supplements, etc., are not always as safe as the labelling and the shops suggest. We have perceived this from the international scientific literature as well as from experimental studies, which several inspectorates requested the Laboratory for the Quality Control of Medicines (LGO) to do on certain commodities, such as Chinese herbs sold as food supplements.

The history and cause of the present research project is as follows. From 1993 onward, the LGO received many samples of Ephedra products, especially from Dutch Health Care Inspectorates (IGZs) and Inspectorates of Health Protection, Commodities and Veterinary Public Health (KvWs), as a result of complaints from the market. These institutes requested the LGO to check whether the products contained certain *synthetic* EP alkaloids instead of the natural, herbal components. The products were suspected of containing especially NPE and EP. These 'slimming' products were popular in the Netherlands in the early ninethies. Meanwhile, other related commodities attained increasing popularity. Ephedra products sold as health products in sports practice and as so-called 'smart products' are still popular. Mainly due to the results found for the slimming products, a joint research project on Ephedra herba was started with the participation of the LGO and the KvW in Maastricht, and in 1998 the KvW in Den Bosch joined in to replace the KvW in Maastricht.

1.2 Purpose and organisation

The principal purpose of this project was to assess whether these Ephedra products can indeed be considered harmless on the basis of the analytical results and the safety reports in the literature. We took into consideration that Ephedra herba contains EP alkaloids, and that these substances may have pronounced effects on the cardiovascular and central nervous systems. A second purpose was to determine whether the products were of herbal origin (Ephedra herba), so, whether they did not contain synthetic substitutes.

The investigations consisted of three studies of samples received by the LGO. The samples had been labelled with one of the scientific or popular names already mentioned and/or 'from natural sources'. Suitable analysis methods, as well as safety and quality requirements, had to be selected. The information required to make this selection was attained through literature studies and is summarised here.

1.3 Chemical and physical properties of ephedrine alkaloids and pharmaceutical quality standards

The major active substance in Ephedra herba is EP (2-methylamino-1-phenylpropan-1-ol). The molecule possesses two chiral centres, so that four stereoisomers are theoretically possible *(d*-ephedrine, *l*-ephedrine, *d*-pseudo-ephedrine, and *l*-pseudo-ephedrine). Other naturally occurring EP-like alkaloids in Ephedra herba are NE and ME. Four stereoisomers are possible for each of these alkaloids as well. In theory, the three EP alkaloids can exist as 12 stereoisomers in total [4] Figure 2, Appendix 2 shows the molecular structures of these three EP alkaloids in their six naturally existing diastereomers. Tables 2 – 4, Appendix 2 show some of the chemical and physical properties of the naturally occurring EP alkaloids and synthetic analogues [5- 7] .Where possible, reference is made to pharmacopoeial monographs. The monographs define and characterise these substances in detail; strict limits are formulated for assay and content of impurities, as some of these substances are intended for clinical use in the preparation of medicinal finished products: racemic EP, chiral pure (-)-EP, chiral pure (+)-PE, and racemic NE [8-10].

1.4 Plant sources of Ephedra herba, composition, and pharmaceutical standards

The plant sources are *Ephedra sinica Stapf* and other EP-containing species of the genus *Ephedraceae*. Examples: *E. sinica Stapf, E. intermedia, E. equisetina Bunge, E. distachya L*. EP alkaloids have also been reported [3, 12] in *Taxus baccata L*, the Indian *Sida cordifolia L*. (*Malvaceae*), *Roemeria refracta D.C.* (*Papaveraceae*) and *Aconitum napellus L*. NPE and NE are also present in khat (*Catha edulis Celastraceae*). These plants mainly grow in Asian countries and South America, except khat, which grows in Africa [3] .

As can be expected for botanical sources, the EP alkaloid content and composition (EP/PE, ME/MPE, and NE/NPE) may vary with the type of plant, sex, time of harvest, and geographical origin[13-16]. The total alkaloid content may vary from 0.5% to 3% [17]. Table 5, Appendix 3 and Figure 3, Appendix 3 present overviews of the composition of EP alkaloids in several Ephedra species according to the literature [12]; Figure 4, Appendix 3 [14] and Table 6, Appendix 3 [14] present the compositions given in the literature of commercial Ephedra herba samples on the Taiwanese market . In a separate validation study, the LGO compared the literature composition data with experimental values (1995). The experimentally determined alkaloid pattern matched well with the literature values for *E. sinica Stapf* (Table 6, Appendix 3), so it is evident that it is well possible to produce Ephedra herba with consistent alkaloid content and ratio.

Compendial quality standards for EP are outlined in Table 7, Appendix 3. The compendial requirements indicate that, in contrast to the pure EP alkaloids, Ephedra herba is much less defined and characterised, but the EP alkaloid patterns in this herb are consistent, with a large range in content and ratio in the several alkaloids.

1.5 Selection of methods

On the basis of the Ephedra herba properties just described and this herb's alkaloids, chromatographic analysis methods were selected from the literature for determinations of the identity and content of the samples [4, 12 - 14, 18 -28], (Sect. 2). Additionally, in studies 2 and 3, the

qualitative compositions given on the label were documented to assess whether other substances that possibly enhance the clinical effects of the EP alkaloids (xanthine derivatives) were present.

1.6 Selection of requirements

Quality. In the literature [4, 12] , ratios and patterns of the sources of Ephedra herba are described on the basis of many samples from a great variety of sources. With the aid of these values, we set requirements for evaluating whether the samples were from natural sources or not (Sect. 2).

Safety. The pharmacological effects of Ephedra herba originate in the EP alkaloids of the herb, which are amphetamine-like compounds. The substances are sympathomimetic drugs, and they stimulate both α and β adrenergic receptors by means of direct and indirect effects [8 - 10], which result in bronchodilation (bronchial muscle relaxation), nasal decongestion (both are peripheric actions due to the release of NE), cardiovascular effects (e.g. hypertension), and peripheric effects. They also act on the central nervous system (CNS). There are some minor differences in effect among the various alkaloids. For example, PE and NE stimulate the CNS less potently and give less hypertension than EP [9 - 11], PE has a weaker effect on blood pressure than EP; NPE stimulates the CNS more than EP (and was therefore used as a very effective slimming agent in the past). Adverse reactions reported for Ephedra herba products are anxiety, restlessness, toxic psychosis, seizures, irregular heart beat, tachycardia, hypertension, skin eruptions, strokes, and death[29 - 35]. The EP alkaloids are included in the doping lists of the International Olympic Committee.

The FDA [29] proposes to reduce risks associated with dietary supplement products containing EP alkaloids by limiting the amount of EP alkaloids and by requiring labelling and marketing measures that give adequate warning and information to consumers. The proposal is to prohibit the marketing of dietary supplements containing 8 mg or more of EP alkaloids per dose and labelling that recommends 8 mg or more in a 6-hour period or a total daily intake of 24 mg or more. The proposal also requires that the label states 'do not use this product for more than 7 days'. It prohibits the use of EP alkaloids with ingredients with a known stimulant effect (e.g. sources of coffeine or yohimbine) that may interact with EP alkaloids. Labelling claims that require long-term intake to achieve the claimed effect (loss of weight, body building) are to be prohibited, as are statements encouraging short-term excessive intake to enhance the claimed effect (e.g. energy). It should also be stated that 'taking more than the recommended serving may result in heart attack, stroke, seizure and death'. This draft law originates from 1997 and is the result of 800 reports of adverse effects associated with Ephedra products on the market in the USA. Due to the adverse advents described by the FDA and other reports, we decided to choose the limit values in this proposal as one of the tools to evaluate safety.

2 Methods and materials

2.1 Types of the products investigated

Study 1 (1993-1996) investigated 81 samples of approximately 35 dietary supplements sold as 'slimming products'. These were provided by several regional IGZs and KvWs. The samples were obtained at a great number of manufacturers and importers, and of some health food stores.

Study 2 (1997-1999) investigated dietary supplements used in sports and smart drugs (often sold as 'herbal energisers' and 'herbal XTC').The samples were provided by several KvWs and by Customs in cooperation with the KvWs. The samples were obtained at manufacturers, importers, and a fitness shop.

Study 3 (1999) investigated smart drugs that KvWs obtained at some manufacturers and importers, and mainly at smart shops. The majority of the samples were provided by the KvW in Den Bosch, obtained at smart shops in Brabant, Limburg, and Amsterdam.

In total, 121 samples from approximately 100 products were investigated in studies 2 and 3.

2.2 Scope of the studies, the sampling plan, and the methodology

There are differences in the scope of the three studies, and the main difference lies between study 1 and the other two. The investigations of study 1 were the result of complaints of the free availability of these products ('slimming products with dangerous herbs') on the Dutch market, which were considered as 'probably illicit'. The aim was to check whether synthetic EP alkaloids were present in the samples, and if so, to determine the concentration. EP and especially NPE were the suspected drugs that were systematically sought for. We also aimed to remove those products containing synthetic alkaloids from the market.

Studies 2 and 3 were a consequence of study 1, which reported 'synthetic' samples. There were also reports from international literature indicating that commodities on the market should preferably not contain EP alkaloids above certain maximum levels and that they are probably unsafe [29 - 35]. In these two studies, the KVWs requested the LGO to determine whether the samples were indeed from natural source as indicated on the label. Study 2 differs slightly from study 3: study 3 was a pilot project focussed on products in smart shops only, the samples in study 2 were obtained from miscelleanous sources.

The procedures for taking samples from the market for these studies - choice of certain products at certain times, choice of manufacturers, importers, and shops, number of samples taken, etc. - are defined in established procedures of the IGZs and KvWs.

Each sample was analysed to see if one or more of the six diastereoisomers of the EP alkaloids was present. The concentration of any such alkaloid (calculated as EP HCl) and the relative composition were determined. In study 1, this whole procedure was followed only if the presence of synthetic EPs had been confirmed. In studies 2 and 3, the qualitative compositions given on the labels were also documented.

The total content and the dosage instructions on the label were used to evaluate whether the sample exceeded the draft safety limits recommended by the FDA (studies 2 and 3). The composition given on the label was used to evaluate whether the sample contained xanthine derivatives added as herbal extracts or as synthetic substances. The name of one or more of the following substances on the label was considered a further risk [3, 29 30, 35]: 'Guarana', 'Kola Nut', 'Gatu Kola', 'caffeine', 'theobromine' and 'theophylline' [3] . The results of the EP alkaloid pattern and ratio were used to assess whether the sample was a product 'of natural, herbal origin' (all three studies).

Evaluation of safety compliance. The primary safety criteria were the FDA draft acceptance criteria [29]: a maximum of 8 mg EP base alkaloids per dose and a maximum of 24 mg daily intake. There was the restriction that these values had to be multiplied by the ratio between the molecular weight of EP HCl and EP base, namely, by 201.7/165.2, giving a maximum of 10 mg (9.8 mg) per dose and a maximum of 30 mg (29.3 mg) daily intake. This is because the contents were determined on the basis of the HCl salts of the alkaloids. Based on these safety criteria, the safety evaluation of the samples were expressed as certain FDA compliance categories, which on their turn were expressed in certain, defined, symbols ('+', '-' etc.). These compliance categories and symbols are defined in Tables 11 and 13, Appendix 6, where the individual safety data are listed.

Evaluation of quality compliance. Definitions of the categories for “natural source” assignment.

- Probable: EP and PE are present in a natural ratio ranging from 0.2 to 15, together with traces of NE, NPE, ME and/or MPE. Category symbol: +.

- Possible: Only EP and PE are present or only traces of EP. Category symbol: ±.

- Probable, but sample enriched with synthetic EP: EP and PE are present in a ratio greater than 15, and traces of NE, NPE, ME, and/or MPE are present. Category symbol: -[1].

- Probable, but sample enriched with synthetic EP alkaloid(s) (e.g. NPE): The synthetic EP alkaloid is present in a therapeutic or almost therapeutic dose, and traces of EP, NE, NPE, ME, and/or MPE are also present. Category symbol: -[2].

- Unlikely: Only EP or another EP alkaloid (e.g. NPE) is present, in an almost therapeutic dose. Category symbol: -.

- Not assigned: No assignment possible as no EP alkaloids could be detected. Category symbol: n.a.

2.3 Analysis methods and materials

2.3.1 General

The LGO Standard Analysis Method for 'Screening of Ephedra analogues' was used in the first and second studies. This method consists of the TLC and HPLC-DAD identification methods. If necessary at low levels, gas chromatography (GC) and an HPLC assay method for determining the contents were also used. The methods originate from various sources [4, 12 - 14, 18 - 27]. With the HPLC method, the six diastereomers, with the exception of ME/MPE, can be separated from each other. A slightly different HPLC-DAD method, with which ME and MPE can be separated [28], was used in the third study. In all studies, the HPLC methods were such that the enantiomers (+ and – forms) of the six diastereomers were not separated; only the diastereoisomers (e.g. EP - PE) were separated.

2.3.2 Reference substances

- (R,S) (-) EP Hydrochloride: Ph.Eur. quality
- (S,S) (+) PE Hydrochloride
- (RS,SR) (±) NE Hydrochloride
- (R,R) (-) NPE Hydrochloride
- (R,S) (-) N-Methylephedrine
- (S,S) (+) N-Methylpseudoephedrine. From Sigma-Aldrich.

2.3.3 TLC

Reagents

- Isopropyl ether, diethyl ether, acetone, tetrahydrofuran, acetaldehyde, ninhydrin, acetic acid (min. 99.8%), bismuth subnitrate, potassium iodide, citric acid, sodium nitrate: all analytical grade, Merck
- Methanol: HPLC grade, Promochem
- Concentrated ammonia R (25%), alcohol R (alcohol 96% v/v): Ph. Eur. quality
- Hydrochloric acid 25% (m/v), analytical grade, Merck, hydrochloric acid 4 M and hydrochloric acid 0.1 M, dilutions made from 25% and 4 M
- Water: demineralised

Other materials

- Thin layer: ready made plates Silicagel 60, F254 (Merck, Art. 5715)
- Ultraviolet (UV) viewing cabinet (254 and 366 nm)
- Micropipets 10 µl.
- TLC developing tank

Mobile Phase

- Isopropylether 60 ml, diethylether 12 ml, acetone 8 ml, tetrahydrofuran 20 ml, acetaldehyde 1.5 ml, ammonia 25% solution 3 ml. All solvents were mixed in a separator funnel with the ammonia solution added last. After the solvent had stood for at least 1 hour (crystallisation may occur), the lower layer was discarded. The upper layer was put in a flask and shaken. The flask was allowed to stand for about 30 minutes. When it was certain that crystals were present and the solvent was practically clear, the solvent was carefully poured into the developing tank.

Spray reagents
- 0.3% (m/v) ninhydrin solution in a mixture of alcohol R + 3% (v/v) acetic acid
- Dragendorff's reagent (Potassium iodo bismuthate reagent)

Reference solutions
- Stock solutions: 100.0 mg of each of the hydrochloric acids of NE, NPE, EP, PE, and ME base were dissolved in water, and the respective solutions were diluted to 10.0 ml with water.
- Reference solutions, individual. 1.0 ml of each stock solution was poured into a volumetric flask, and each solution was diluted to 10.0 ml with methanol (concentrated at 1 mg/ml).
- Reference solution, mixture: 1.0 ml of each stock solution of NE, NPE, EP, and PE was poured into a volumetric flask, and this was diluted to 10.0 ml with methanol (concentrated at 1 mg/ml).

Test solutions
- Tablets/capsules. A powdered tablet, or the content of one capsule, was put into a flask, 2 ml of water was added, the mixture was shaken and heated gently in a water bath. Then 8 ml of methanol was added, and the mixture was shaken for 15 minutes. The mixture was filtered.
- Drops and solutions. 1.0 ml was diluted to 10.0 ml with methanol.

Chromatogram development
We applied 10 µl of each reference solution and 2, 5, and 10 µl of the test solution to the plate separately. In the case of liquid samples, 1 and 5 µl were also applied directly. The chromatogram was developed over a path of 15 cm. The plate was allowed to dry at 100° C - 105° C (5-10 minutes) and was afterwards examined in UV light at 254 nm and at 366 nm. The plate was sprayed with ninhydrin solution, then heated to 110°C for about 5 minutes till the optimum colour was acquired. Another developed plate was sprayed with Dragendorff's reagent to detect ME and MPE. The plate was successively sprayed with a 1% (m/v) solution of sodium nitrite in water to enhance the sensitivity.

If a spot in the chromatogram obtained with the test solution corresponded to one of the reference substances, this was verified by cochromatography (a mixture of test solution and reference candidate). The concentrations of the test and reference solutions should be equivalent. The test is not valid unless the chromatogram obtained with the reference solution shows four clearly separated principal spots (NE, NPE, EP, PE).

2.3.4 HPLC 1

Reagents

- Potassium dihydrogen phosphate, hexylamine, phosphoric acid 85%: all analytical grade from Merck.
- Acetonitrile: HPLC grade from Promochem
- Sodium hydroxide 2M = sodium hydroxide, dilute R: Ph.Eur. quality
- 0.1 M Sodium hydroxide = 20 times diluted sodium hydroxide 2M.
- Water: demiwater

Phosphate buffer solution

- 20 mM Potassium dihydrogen phosphate + 0.2% v/v hexylamine set at pH = 4.0 with phosphoric acid 85%. The solution was filtered through a membrane filter of 0.45 µm.

Other materials

- Solid phase extraction (SPE): Sep-pak C18 cartridges from Waters
- Column: Lichrospher RP Select-B, 5 µm. Dimensions (L*ID) 125 mm * 4.0 mm, guard column: 4.0 cm * 4.0 mm (L* ID) with the same material (Merck).

Reference solutions

- Reference solution: combinations of NE, NPE, EP, PE, and ME in concentration ranges from 0.01 mg/ml – 1.0 mg/ml.
- System suitability mixture: 20.0 mg of the hydrochloric acids of NE, NPE, EP, PE, and ME base were dissolved in 15 ml of phosphate buffer solution, with gentle heating in a water bath if necessary, and after cooling, the solution was diluted to 20.0 ml with phosphate buffer solution.

Test solutions

- Tablets/capsules. A suitable quantity of powdered tablets or capsules was weighed and put in a volumetric flask, then 6 ml of phosphate buffer solution was added. The mixture was then gently heated in a water bath for at least 5 minutes, then shaken for 15 minutes and diluted to 10.0 ml with phosphate buffer solution. The mixture was filtered before an extraction was performed. The procedure was repeated for another quantity of the same sample.
- Drops and solutions: 1.0 ml of sample was diluted to 10.0 ml with phosphate buffer solution. Afterwards, an extraction was performed. The procedure was repeated for the same sample.

SPE

1. The SPE cartridge was activated with 10 ml of methanol.
2. The cartridge was then conditioned with 10 ml water, followed by 10 ml of 0.1 M sodium hydroxide.
3. Then 2.0 ml of the sample solution and sufficient 2 M sodium hydroxide to reach a pH of at least 13 were added to the cartridge.
4. The cartridge was washed with 3 ml of water.
5. The cartridge was eluted with 3 ml of methanol. The eluent was collected and diluted to 10.0 ml with phosphate buffer solution.

Equipment
- Waters HPLC-DAD combination; pump type 600 MS, autosampler WISP 717, and diode array detector 996.
- Software: Millenium[32] Chromatography Manager.
  Operating conditions: column temperature: 25° C, injection volume: 20 µl, detection: 257.2 nm, wavelength range: 230-280 nm, spectral resolution: 1.2 nm, data rate: 1.0 spectrum/second.

Mobile phase
- Isocratic conditions with phosphate buffer solution, at a flow of 1.00 ml/min.
- The gradient was effected when samples were injected directly without sample clean up. After 9 minutes, a linear gradient was made: in 4 minutes to 15% (v/v) acetonitrile and 85% (v/v) phosphate buffer solution, which was continued for 5 min. If needed in the case of sample matrix effects, a linear gradient was started next at 15 minutes to 50% (v/v) acetonitrile and 50% (v/v) phosphate buffer solution in 5 minutes, and was continued for 5 minutes.

System suitability test
A system suitability test was performed at regular stages. The following criteria are to be met:

Ephedrine analogue	Retention time (seconds)	RT relative to EP	Resolution	UV maximum (nm)		
				Maximum 1	Maximum 2	Maximum 3
NE	165 ± 25	0.70 ± 0.05	> 1.0	258 ±3	252 ± 3	264 ± 3
NPE	200 ± 25	0.82 ± 0.05	> 1.0	258 ±3	252 ± 3	264 ± 3
EP	250 ± 50		> 1.0	258 ±3	252 ± 3	264 ± 3
PE	270 ± 50	1.15 ± 0.05	> 1.0	258 ±3	252 ± 3	264 ± 3
ME	295 ± 50	1.27 ± 0.05	> 1.0	258 ±3	252 ± 3	264 ± 3

Identification
The extracted test solution and reference solution were injected, and the respective chromatograms were examined. If a peak in the chromatogram obtained with the test solution corresponded to one of the reference substances, this was verified by cochromatography, and peak purity was then ascertained. For this, the concentrations of the test and reference solutions had to be equivalent or had to be made so.

The identification was considered positive if:

1. The retention time of reference, sample, and the mixed sample/reference peak differed by less than 5%.
2. The peak purity of the mixture sufficed if purity angle was less than the purity threshold (for Waters systems).
3. The difference between the UV maxima of the reference and test-solution spectra was less than 2 nm.
4. The spectral library match of the test solution with a candidate sufficed if the match angle was less than the match threshold (for Waters systems when the spectra of references and sample were recorded under the same conditions).

Remark: The UV spectra of EP analogues are closely related; only the fourth derivative spectra gave small differences between EPs/PEs (Figure 5, Appendix 4).

Assay
For assay determination, the concentrations of the test and reference solutions had to be equivalent or to be made equivalent. The test solutions were injected twice. The reference solutions were injected frequently, between the injections of the samples. A mean response factor for each alkaloid standard was calculated as the area divided by the concentration. The content of each alkaloid, except ME and MPE, was calculated as the hydrochloric acid salt.

2.3.5 HPLC 2

HPLC 2 was applied to sample nos. 4637 - 4727 of study 3 and samples nos. 5019 and 5020 of study 2.

In comparison with the test procedure described in Sect. 2.3.4., in HPLC 2 the column and composition of the mobile phase were changed to allow a quantitative determination of MPE in the presence of ME. The test results obtained by HPLC 2 are therefore more accurate with respect to the concentration of ME.

Reagents
- Sodium acetate trihydrate: analytical grade, Merck.
- Acetic acid glacial 100%: extra pure, Merck
- Triethylamine: analytical grade, Baker
- Acetonitrile: HPLC grade from Promochem
- Sodium hydroxide 2M = sodium hydroxide dilution, Merck
- 0.1 M Sodium hydroxide = 20 times diluted sodium hydroxide 2M.
- Water: demineralised water

Acetate buffer solution
- 0.1 M Sodium acetate trihydrate + 0.3 % v/v triethylamine set at pH 4.8 with acetic acid glacial 100 %. The solution was filtered through a membrane filter of 0.45 µm.

Solvent
Dilute the acetate buffer five times with water.

Other materials
- SPE: Sep-pak C18 cartridges from Waters
- Column: YMC pack Phenyl, 5 µm. Dimensions (L*ID) 250 * 3.0 mm, guard column: phenylpropyl, 4.0 cm * 3.0 mm (L*ID).

Reference solutions
- The reference solutions were combinations of NE, NPE, EP, PE, ME, and MPE in concentration ranges from 0.02 mg/ml – 0.4 mg/ml.
- For the system suitability mixture, 4 mg of synephrine (SYN) and 20.0 mg of the hydrochloric acids of NE, NPE, EP, PE, ME base, and MPE base were dissolved in 80 ml of solvent solution, with gentle heating if necessary, and after cooling the solution was diluted to 100.0 ml with solvent.

Test solutions

- A suitable quantity of powdered tablets or capsules was weighed and put in a volumetric flask, and 6 ml of solvent solution was added. The mixture was then gently heated for at least 5 minutes, then it was shaken for 15 minutes, diluted to 10.0 ml with solvent, and filtered before extraction. The procedure was repeated for another quantity of the same sample.
- Drops and solutions were diluted with 1.0 ml of sample to 10.0 ml with solvent. Afterwards, an extraction was performed. The procedure was repeated for the same sample.

SPE

1. The SPE cartridge was activated with 10 ml of methanol.
2. The cartridge was conditioned with 10 ml water, and then 10 ml of 0.1 M sodium hydroxide.
3. Two millilitres of the sample solution was added to the cartridge, then sufficient 2 M sodium hydroxide to reach a pH of 13 was added.
4. The cartridge was washed with 3 ml of water.
5. The cartridge was eluted with 3 ml of methanol. The eluent was collected and diluted to 10.0 ml with solvent.
6. The solution was placed in an ice bath for 15 minutes. The cold solution was filtered.

Equipment

- Waters HPLC-DAD combination; Alliance 2690 separation module and DAD detector 996.
- Software: Millenium[32] Chromatography Manager.
  Operating conditions: column temperature: 25° C, injection volume: 20 µl, detection : 257 nm, wavelength range: 230-280 nm, spectral resolution: 1.2 nm, data rate: 1.0 spectrum/second.

Mobile phase

- Isocratic conditions with 4% acetonitrile and 96% acetate buffer solution, at a flow of 0.80 ml/minutes.
- Gradient was effected when samples were injected directly without sample clean up.After 12 minutes, a linear gradient was made: in 4 minutes to 15% (v/v) acetonitrile and 85% (v/v) acetate buffer solution, which was continued for 2 minutes. A linear gradient was started next at 18 minutes to 50% (v/v) acetonitrile and 50% (v/v) acetate buffer solution in 5 minutes and was continued for 5 minutes.

System suitability test

A system suitability test was performed at regular stages. The following criteria are to be met:

Ephedrine analogue	RT relative to EP	Resolution	Peak symmetry	UV maximum (nm)		
				Maximum 1	Maximum. 2	Maximum 3
SYN	0.40 ± 0.05		< 2.0	272 ±3		
NE	0.71 ± 0.05	> 1.0	< 2.0	257 ±3	251 ± 3	262 ± 3
NPE	0.81 ± 0.05	> 1.0	< 2.0	257 ±3	251± 3	262 ± 3
EP	1	> 1.0	< 2.0	257 ±3	251 ± 3	262 ± 3
PE	1.14 ± 0.05	> 1.0	< 2.0	257 ±3	251 ± 3	262 ± 3
ME	1.33 ± 0.05	> 1.0	< 2.0	257 ±3	251 ± 3	262 ± 3
MPE	1.44 ± 0.05	> 1.0	< 2.0	257 ±3	251 ± 3	262 ± 3

Identification
See Sect. 2.3.4.

Assay
See Sect. 2.3.4.

2.3.6 Validation of the TLC method

Identification

- Specificity: Figure 5, Appendix 4 shows representative thin-layer chromatograms of reference and sample solutions. The spots of the several EP diastereomers are separated sufficiently; excipients do not interfere.
- Detection limit:1µg.

2.3.7 Validation of HPLC 1 and 2

Identification

- Specificity: Figure 6, Appendix 4 shows representative HPLC 2 chromatograms of reference and sample solutions. The peaks of the several EP diastereomers are separated sufficiently; excipients do not interfere.
- Limit of spectral identification: 0.05 mg /ml (1.0 µg on the column).

Assay

- The linearity has been validated in the range 0.01 – 2.0 mg/ml for HPLC method 1 and 0.02 – 0.4 mg/ml for HPLC method 2 ($r^2 > 0.99984$; $n = 5$, visual inspection at random distribution residual variations). During the analysis of the samples within the day and day to day, reference samples of several strengths were also analysed, and we checked whether the assay of the reference remained within 98%-102% of the starting value.
- Limit of quantification: 0.01 mg/ml (0.2 µg on the column).
- Limit of detection: 0.005 mg/ml
- Accuracy: ≥ 97 % recovery
- Precision: during the analysis of the samples within the day and day to day, reference samples of several strengths were also analysed, and we checked whether the assay of the reference remained within 98%-102 % of the starting value. Intermediate precision was estimated as approximately 2%.
- Specificity: same as the 'Identification' specificity.

The TLC and HPLC methods can be considered sufficiently validated for the intended use.

3 Results

3.1 Description of the samples

Table 8, Appendix 5 is an overview of the pharmaceutical dosage forms of a part of the studied samples and the labelled active ingredients. It shows that the dosage forms are mainly capsules, tablets, dry herb stems, powders, chewing gum, and liquids (including drops). These types of products are rarely produced with only Ephedra or EP alkaloids, but mostly in combination with several other active ingredients, which, by definition, form a potential hazard.

3.2 Quality of the samples

Note: Table 9,Appendix 5 only presents the results of the samples that do not comply with the label “natural origin”; results for the other samples in this study have not been included due to the scope of the study.

Eighty-one samples from approximately 35 products were investigated in study 1 (Table 9, Appendix 6). The table shows three samples in the category 'probably of natural origin' (+) or 'not assigned' (n.a.): this was the result of manufacturer's changes in composition of the products at the request of the Inspectorate. All the other samples were samples in the categories 'unlikely of natural origin', or 'of natural origin enriched with a synthetic analogue' (-, -[1] or -[2]).

Study 2 (Table 10, Appendix 6)

	Number	Percentage
- Samples probably of natural origin (+)	31	64.5
- Samples possibly of natural origin (±)	9	18.8
- Samples unlikely of natural origin, or of natural origin enriched with a synthetic analogue (-, -[1] or –[2])	6	12.5
- Samples of category 'not assigned' (n.a.)	2	4.2
- Total	48	100

Study 3 (Table 11, Appendix 6):

	Number	Percentage
- Samples probably of natural origin (+)	41	56.2
- Samples possibly of natural origin (±)	12	16.4
- Samples unlikely of natural origin or of natural origin enriched with a synthetic analogue **(-,** -[1] or -[2]).	11	15.1
- Samples of category 'not assigned' (n.a.)	9	12.3
- Total	73	100

3.3 Safety of the samples

Summary of the safety results in study 2 (Table 12, Appendix 6):

- Samples investigated	48
- Samples within FDA range (+)	10
- Samples exceeding FDA range (-)	38
- Samples containing a xanthine derivative	17
- Samples that exceeded FDA range (-) and contained a xanthine derivative	12

Summary of the safety results in study 3 (Table 13, Appendix 6):

- Samples investigated	73
- Samples within FDA range (+)	18
- Samples exceeding FDA range (-)	55
- Samples containing a xanthine derivative	19
- Samples that exceeded FDA range (-) and contained a xanthine derivative	16

Note that, with respect to the assessment of FDA compliance and safety, the calculated values (Tables 11, 13, Appendix 6) for 'maximum daily dosage' of the samples that were not labelled with a daily dose, but only a dose per serving, present a rather 'flattering' view of the situation and may, in practice, be greater than indicated in the tables. For example, for sample 4343 in Table 13, Appendix 6, the recommended dosage is 2 tablets per serving and no daily dose is recommended. The 'maximum daily dose' is calculated as 7 mg, but *may* be more; the FDA requirements *may* still be exceeded.

3.4 Summary of the results in totality

Table 9, Appendix 56 (study 1) is not considered for quality and safety risk evaluation due to the scope of the investigation, but shows that approximately 30% of the 81 samples of the 35 products contained synthetic NPE or EP.

In studies 2 and 3 (Tables 10-13, Appendix 6), 17 of the 121 samples of the 100 products were not likely of natural origin. Ninety-three of these samples were outside the FDA range, 36 samples contained a xanthine derivative, and 28 samples that were outside the FDA range contained a xanthine derivative.

4 Discussion

Significance of the results. First we evaluate the significance of the results with respect to the following points.

•Regarding the sampling plan, to what extent are the samples in the pilot studies representative of products for sale on the Dutch market? The samples in study 1 are not representative, the results are only presented to indicate the cause for the further studies (2 and 3). Studies 2 and 3 are more representative, but it should be born in mind that the sampling for study 3 was deliberately aimed at those products (smart shops!) suspected of large doses of Ephedra. However, such products were and indeed still are for sale.

•In the safety analysis, the *total* EP alkaloid content was calculated with respect to EP HCl, although in fact six botanical EP alkaloid diastereomers (EP, PE, NE, NPE, ME, and MPE) with two enantiomers for each diastereomer exist. In theory, there are only slight differences in activity, and determining the total alkaloid content is, in our view, permissible. The literature also uses total content determinations. [29, 31, 36] .

•Is there a difference in the pharmacokinetic behaviour of Ephedra extracts and pure EP alkaloids? Gurley and Gardner [37] compared synthetic EP and Ma Huang, and the pharmacokinetic absorption parameters were similar. They conclude that there are no absorption differences between the herb and the synthetic analogues, and the toxic effects were due to an overdose of the natural extract itself.

•The acceptance criteria for determining 'natural origin' are based on the overall values found in the literature for the natural patterns and ratios of EP alkaloids in several Ephedra species. Thus, they can be considered reliable. The FDA draft safety criteria are based on 800 reports of adverse effects and the FDA is an established regulatory institute, known world wide.

Consequences of the safety risk evaluation. We are aware that the FDA draft criteria (single dose: a maximum of 10 mg calculated as EP HCl; daily dose: a maximum of 30 mg) seem rather strict when we compare them with the doses used in medical practice: 30 mg - 60 mg EP HCl daily [8 - 10] and a single dose of 15 mg -30 mg total EP HCl in Ephedra herba [2]. The recommended *maximum* daily dose on the labelling of over the counter (OTC) EP medicinal products (cough syrups) is 35 mg - 40 mg EP HCl, which is not far from the FDA permitted daily dose (30 mg alkaloids as EP HCl). These OTC products fall under the Medicines Act, and thus the manufacturers must show that they are safe *before* they are marketed, but this obligation does not exist for commodities.

A recent, independent review of more than 800 adverse events in the USA [31] seems to support the FDA draft requirements. It has also been shown that xanthine derivatives [found in 30% of the samples (studies 2 and 3)] potentiate the effects of EP alkaloids [3, 29, 20, 35]. Beltman et al. [3] of the National Poisons Control Centre (NVIC), RIVM, give general advice regarding the toxicological risk of products containing Ephedra on the basis of the labelling information and data from handbooks on medical use and toxicological studies with rats. They conclude that a daily dose of 50 mg -150 mg of EP alkaloids is recommended on the labelling of most of the Ephedra capsules studied and that these doses can cause unwanted

effects. Our experimental results make it evident that the doses are even greater in reality (Tables 11, 13, Appendix 6), daily doses up to 210 mg are possible. Add to this the potential increase in hazard if the products contain xanthine derivatives and/or other effect-enhancing substances.

Consequences of the quality evaluation. Fourteen percent of the samples in studies 2 and 3 were not of natural origin, which indicates that these products are not being produced by adequate and constant manufacturing methods. Adulteration of these products with undeclared agents must also be taken into account. The Ephedra herba extract used in the production of these products can vary significantly with respect to the composition of the six possible EP alkaloids and the total content, due to differences in extraction methods, parts of the plant used for extraction, harvest time, country, etc. [1, 3, 20, 35]. The variations in content among the products and within one product contribute to significant variations in their potency. Furthermore, the amounts of potential toxic components (herbicides, insecticides, heavy metals, etc.) in the products should be known and controlled.

Possibilities for improvement of quality and safety.

-*Consideration from a scientific point of view.* The quality and safety results show the need for improved quality control by the industry. Product dossiers, with descriptions of compositions, manufacturing processes, control tests etc., and control of these processes by means of either organised self-inspection by the manufacturers or, preferably, inspection by government authorities are needed.

We recommend improving the existing pharmacopoeial quality monographs for Ephedra herba (e.g. by tightening the requirements for potency and adding limits for toxicological plant components [herbicides etc.]). We would welcome a Ph. Eur. monograph for Ephedra herba containing adequate tests for all important parameters. This would be a useful quality standard tool for the industry. Recommendations for quality standards for the *finished products* prepared from Ephedra herba (oral liquids, tablets, capsules etc.) will then also be needed[38] . Halkes et al. [38] present a set of parameters to assess the quality of products put on the market as food supplements; this can be a useful tool as well.

-*Considering national legislation.* The legislation regulations and acts for public health that could possibly apply to Ephedra products are rather complicated. The following national regulations and acts are considered.

• The Dutch legislation on prevention of the misuse or abuse of chemicals is based on European legislation (EEG Nos. 3677/90 and 92/109/EEG). It is applicable to precursors. EP and PE, whether natural or not, are on list 1 of this act because they can be used in the synthesis of methamphetamine. To be able to trade or possess materials on list 1, both suppliers and customers must have permits. The Central Licensing Office for Imports and Export, Tax and Customs Administration (CDIU) issues permits, which the Economic Investigation Agency of the Ministry of Economic Affairs supervises. However, this legislation applies to the active substances EP and PE, but not the finished products prepared from these substances.

•The Dutch Medicines Act, based on European legislation 65/65/EEG, applies to medicinal products, but not to the Ephedra products. Registered medicinal products containing EP

alkaloids are cough syrups, a product for injection against bronchospasms and certain forms of hypotension, and they contain synthetic active substances: EP hydrochloride or EP sulphate. The products are Famel efedrine HCl stroop, 1.3 mg/ml [registration number RVG 00378]; Abdijsiroop [RVG 02324]; Bronchicum Extra Sterk [RVG 03730]; and Efedrine HCl injectievloeistof 50 mg/ml PCH [RVG 51937] [39]. The only products registered with Ephedra herba as the active substance (natural source) are the homeopathic products *Ephedra vulgaris* druppelvloeistof [RVH 80647] and *Ephedra vulgaris* granules [RVH 91507], but these products are registered on the basis of different requirements than regular medicinal products are. There is less focus on efficacy, but more on strict requirements for safety and quality, as is the case for homeopathic products in general in the Netherlands.

• Food legislation is a part of the Dutch Commodities Act. Herbs, herbal tea, herbal soft drinks, alcoholic beverages, etc., fall under this legislation, as do medicinal dosage forms like capsules, tablets, etc., prepared from herbs or herbal extracts, whether mixed with excipients or not. This legislation is therefore also applicable to the Ephedra products.

Legislation has recently been prepared specifically for herbs: the Herbal Preparations (Commodities Act) Decree [38]. This consists of several lists with restrictions for specifically mentioned herbs. It also lists herbs that may not be used in commodities, in view of public health , such as 'Aristolochia'. It is remarkable to see that Ephedra Herba is not taken up in this list. The possible complications of its use are comparable to that of herbs listed in Annex III, f.i. Datura stramonium or Digitalis purpurea.

-*Considering international legislation.* At present, there is no international legislation applicable to Ephedra products on the Dutch market that would improve their quality and safety. A first draft of a 'Directive on Traditional Medicinal Products' has recently been prepared by the British health authorities for the European Commission, but this is not yet applicable.

We conclude that the possibilities for improving the safety and quality of the Ephedra herba products on the Dutch market are complex, considering the existing legislation regulations and acts. Nevertheless, this project shows that there is certainly a need for improvement.

Recommendation. We recommend treating these products as medicinal products or as commodities to which restrictions must be applied.

5 Conclusion

The principal purpose of the project was to assess the safety risk of products containing Ephedra herba. The results of the experimental studies show that the content of the active substances in the products often exceeded acceptable values, and that combinations of these substances with xanthine derivates were present in the products. These derivatives are known to potentiate the effects of the active substances, including unwanted effects. The products were often not of herbal origin as suggested by the labelling, and the quality of Ephedra herba in these products fluctuated. The first observation is a concern from a toxicological point of view, the second is a concern for the quality aspect. This project shows that there is a need for improvement of safety and quality of these products, in view of public health.

References

1. Deutscher Apotheker Verlag Stuttgart, editor. Deutsches Arzneibuch. 1999; monograph Ephedrakraut.

2. Hansel R., Keller K., Rimpler H., Schneider G. Hagers Handbuch der Pharmazeutischen Praxis, Part 5: Drogen E-O. 5th ed. Berlin: Springer; 1993; 46-57

3. Beltman W, Riel AJHP van, Wijnands-Kleukers APG, Vriesman MF, Hengel-Koot IS van den, Vries I de, Meulenbelt J. Smartshops. Overzicht van producten, geclaimde werking en hun medisch-toxicologische relevantie. Bilthoven, The Netherlands: National Institute of Public Health and the Environment (RIVM) 1999; Report no. 348802017.

4. Betz J M. Alkaloids of Ma Huang (Ephedra spp.). Letter to LGO, november 5, 1996 (unpublished data).

5. (+) Norpseudoefedrinehydrochlorid, Deutscher Arzneimittel Codex (DAC) 1986; Part 2. Vol 87, page N-170.

6. D,L Norpseudoefedrinhydrochlorid, Kommentar zum Arzneibuch der DDR. Vol 7; 1966.

7. D,L-Cathine hydrochlorid, Deutsches Arzneibuch 2. Arzneibuch der DDR (AB-DDR) 83; 1985.

8. Goodman LS. Goodman and Gilman's The pharmacological basis of therapeutics. 8th ed. New York: McMillan 1993; 169-170.

9. Reynolds JEF, editor. Martindale, The Extra Pharmacopoeia. 31st ed. London: The Pharmaceutical Press 1996; 1575-1588

10. Informatorium Medicamentorum, Union of Pharmacists (KNMP), KOMBI/rom, 's Gravenhage, February 1999.

11. Bosch JA, Pennings EJM, Wolff de FA, Psycho-actieve paddestoel- & plantproducten; toxicologie en klinische effecten. Leiden: Ministry of Public Health, Welfare and Sports (VWS) Report 1997.

12. Betz JM, Gay ML, Mossoba MM, Adams S. Chiral gas chromatographic determination of ephedrine-type alkaloids in dietary supplements containing Ma Huang. JAOAC 1997; 80 (2): 303.

13. Ying-Mei L, Shuenn-Jyi S. Determination of ephedrine alkaloids by capillary electrophoresis. J Chromatogr 1992; 600: 370-2.

14. Ying-Mei L, Shuenn-Jyi S, Shiow-Hua C, Hsien-Chang C, Yuh-Pan C. A comparative study on commercial samples of Ephedrae herb. Planta Med 1993; 59: 376.

15. Tanaka T, Ohba K, Kawara K, Sakai E. Comparison of the constituents of Ephedra herbs from various countries on ephedrine type alkaloids. Nat Med 1995; 49(4): 418.

16. Kondo N, Mikage M, Idaka K. Medico-botanical studies of Ephedra plants from the Himalayan region, part III: Causative factors of variation of alkaloid content in herbal stems. Nat Med 1999; 53 (4): 1340-3443.

17. Hartke K, Mutschler E, Rucker G, editors. Kommentar zum Deutsches Arzneibuch. Nordlingen: 1999.

18. Zhang, Yaowu-Fenxi-Zazhi. Detection and identification of the alkaloids in herb ephedrae (ma-huang) by chemical tests and HPTLC. Anal Abstr 5505G136 1992; 12(1): 38-41.

19. Longo M, Martines C, Rolandi L, Cavallaro A. Simple and fast determination of some phenetylamines in illicit tablets. J Liq Chrom 1994; 17(3): 649-58.

20. Imaz C, Carreras D, Navajas R, Rodriguez C, Rodriguez A F, Maynar J, Cortes R. Determination of ephedrines in urine by HPLC. J Chromatogr 1993; 631: 201-5.

21. Zhang Jian, Tian Zhen, Lou Zhi-cen. Simultaneous determination of six alkaloids in Ephedrae herb [Ephedra] by HPLC. Planta Med 1988; 54: 69.

22. Flurer CL, Lin LA, Satzger RD, Wolnik KA. Determination of ephedrine compounds in nutritional supplements by cyclodextrin-modified capillary electrophoresis. J Chromatogr 1995; 669: 133-9.

23. Merwe PJ van der, Brown LW, Hendrikz SE. Simultaneous quantification of ephedrines in urine by high-performance liquid chromatography. J Chromatogr B, 1994; 661: 357-61.

24. Lurie IS. Application of capillary electrophoresis to the analysis of seized drugs. Int Lab 1996; 21-9.

25. Herraez-Hernandez R, Campins-Falco P, Totajada-Genaro LA. Determination of amphetamine and related compounds using chloroformates for derivatization and high-performance liquid chromotography. Analyst 1998; 123: 2131-7.

26. Shuenn-Jyi S. Identification by chemical analysis of the botanical sources of commercial samples of Chinese herbal drugs, J Food Drug Anal1997; 5(4): 285.

27. Starmer GA. Analysis for drugs in saliva. Canberra: Federal Office of Road Safety 1994.

28. Hurlbut JA, Carr JR. Solid-phase extraction cleanup and liquid chromatography with ultraviolet detection of ephedrine alkaloids in herbal products. JAOAC 1998; 81 (6): 1121-7.

29. FDA proposes safety measures for ephedrine dietary supplements. U.S. Department of Health and Human Services, http://www.fda.gov, HSS News P97-15, June 2, 1997.

30. Gurley BJ, Gardner SF, Hubbard MA. Content versus label claims in ephedra-containing dietary supplements. J Health Syst Pharm 2000; 57: 963-9.

31. Halle CA, Benowitz NL. Adverse cardiovascular and central nervous system events associated with dietary supplements containing Ephedra alkaloids. N Engl J Med *in press*.

32. Zaacks SM, Klein L, Tan CD, Rodriquez ER, Leikin JB. Hypersensitivity myocarditis associated with Ephedra use. J Toxicol Clin Toxicol 1999; 37 (4): 485-9.

33. Fisher CR, Veronneau SJ. Herbal preparations: a primer for the aeromedical physician. Aviation Space Environ Med 2000; 71 (1): 45-60.

34. Ko RJ. Causes, epidemiology, and clinical evaluation of suspected herbal poisoning. J. Toxicol Clin Toxicol 1999; 37 (6): 697-708.

35. Young R, Gabryszuk M. Ephedrine and caffeine mutually potentiate one another's amphetamine-like stimulus effects. Pharmacol Biochem Behav1998; 61: 169-73.

36. Kernan WN, Viscoli CM, Brass LM, Broderick JP, Brott T, Feldmann E, Morgenstern LB, Wilterdink JL, Horwitz RI. Phenylpropanolamine and the risk of hemorrhagic stroke. N Engl J Med *in press*.

37. Gurly BJ, Gardner SP. Ephedrine pharmacokinetics after the ingestion of nutritional supplements containing *Ephedra sinica* (ma huang). Ther Drug Monit 1998; 20: 439-45.

38. Halkes SBA, Meer JH van, Woerdenbag HJ, Jans AL, Tome SF des, Kuy A van der. Teelt en oogst beter controleren. Kwaliteit, veilgheid en werkzaamheid van plantaardige medicinale bereidingen. Pharm Weekbl 2000; 135 (34): 1260-5.

38. DATHUG (Databank Humane Geneesmiddelen). Electronic database of the Medicines Evaluation Board in the Netherlands (MEB). www.cbg-meb.nl

39. Warenwetbesluit Kruidenpreparaten, Staatsblad 2001; 56: 's Gravenhage, 31 January 2001

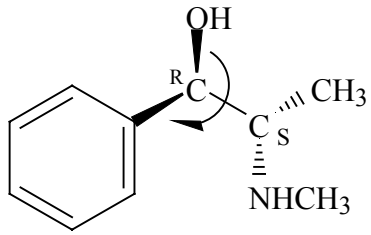
Appendix 1 Synonyms for Ephedra herba

Table 1 Popular names for Ephedra herba [3]

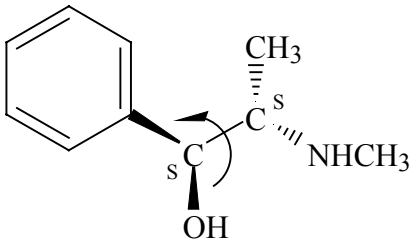
Arizona jointfir	Desert tea	Nevada jointfir
Ask-for-trouble	Green ephedra	Popotillo
Bringham tea	Horse tail	Sand cherry
Bringham young weed	Jointfir	Sea grape
Bringham weed	Longleaf jointfir	Somalata (Sanskriet for “Moon tea”)
California jointfir	Ma Huang/Hwang (Chinese)	Squaw tea
Canutillo	Mexican tea	Stick tea
Cay note	Miner’s tea	Tapopote
Chinese ephedra	Mormon tea	Teamsters’ tea
Clokey’s jointfir	Mtshe (Tibet)	Whorehouse tea
Death Valley ephedra	Narom (Pakistan)	Zeedruif
	Nevada ephedra	

Figure 1 Ephedra herba, dried

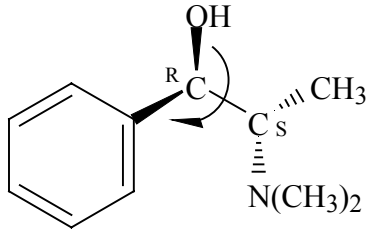
Appendix 2 Existing Ephedrine analogues, reported physical properties and quality monographs



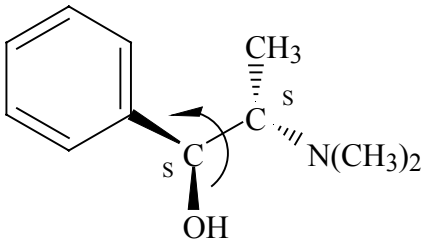
(-)-Ephedrine



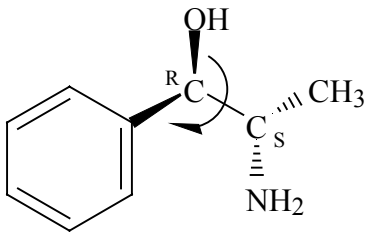
(+)-Pseudoephedrine



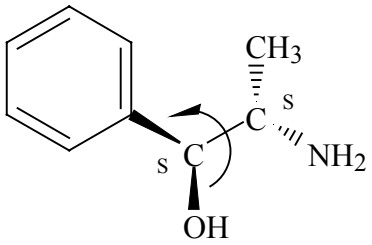
(-)-N-Methylephedrine



(+)-N-Methylpseudoephedrine



(-)-Norephedrine



(+)-Norpseudoephedrine

Figure 2 Moleculair structures of the ephedrine analogues [4]

Table 2 Reported properties and quality of 2-methylamino-1-phenylpropan-1-ol isomers

	Relative molecular mass	Melting point (degrees C)	Specific rotation (°)	CAS no.	Monograph
(-) Ephedrine (1R,2S) [erythro-α]: **natural form**					
(-) E. anhydrous	165.2	36	-41/-43	299-42-3	Ph.Eur. 0488
(-) E. hemihydrate	174.2	42	-41/-43		Ph.Eur. 0489
(-) E. hydrochloride	201.7	219	-33.5/-35.5	50-98-6	Ph.Eur. 0487
(+) Ephedrine (1S,2R)					
(+) E. hemihydrate	174.2	39-43	+ 40.5	321-98-2	
(+) E. hydrochloride	201.7	218-220	+ 34.3	24221-86-1	
(±) Ephredrine (racephedrine)					
E. hydrochloride, Racemic	201.7	188		134-71-4	Ph.Eur. 0715
(+) Pseudoephedrine (1S,2S) [threo-α]: **natural form**					
(+) Pseudoephedrine	165.2	118-120	+ 52	90-82-4	
(+) P. hydrochloride	201.7	184	+ 61.0/62.5	345-78-8	Ph.Eur. 1367
(-) Pseudoephedrine (1R,2R)					
(-) Pseudoephedrine	165.2	118-120	- 49	321-97-1	

Table 3 Reported properties and quality of 2-amino-1-phenylpropan-1-ol isomers

	Relative molecular mass	Melting point (degrees C)	Specific rotation (°)	CAS no.	Monograph
(-) Norephedrine (1R,2S)-2-amino-1-phenyl-1-propanol): **natural form**					
(-) Norephedrine	151.2	51-53	- 41	492-41-1	
(+) Norephedrine (1S,2R) [erythro-α]-2-amino-1-phenyl-1-propanol)					
(+) Norephedrine	151.2	51-54	+ 40	37577-28-9	
(+) N. hydrochloride	187.7	174-176	+ 33.4	40626-29-7	
(±) Norephedrine (racemic)					
Phenylpropanolamine Hydrochloride	187.7	194-196		154-41-6	Ph.Eur. 0683
(+) Norpseudoephedrine (1S,2S)-2) [*threo-α*] {INN: Cathine}: **natural form**					
(+) Norpseudoephedrine	151.2	77.5-78		2153-98-2	
(+) Norspeudo. hydrochloride	187.7	180-183	+42.5/+44.0		DAC 1986 [5]
(-) Norpseudoephedrine (1R,2R): Metabolite in urine of khat users					
(-) Norpseudoephedrine	151.2	180-183	-41.7	53643-20-2	
(±) Norpseudoephedrine {D,L-cathine hydrochloride}					
(±) Norpseudo. hydrochloride	187.7	169-173		54680-46-5	2.AB-DDR [7]

Table 4 Reported properties and quality of N–methyl-2-methylamino-1-phenylpropan-1-ol isomers

	Relative molecular mass	Melting point (degrees C)	Specific rotation (°)	CAS no.	Monograph
(-) N-Methylefedrine (1R,2S) : **natural form**					
(-) N-Methylephedrine	179.3	86-88	-29.2	552-79-4	
(+) N-Methylephedrine (1S,2R)					
(+) N-Methylephedrine	179.3	87-90	+29	42151-56-4	
(+) N-Methylpseudoephedrine (1S,2S) : **natural form**					
(+) N-Methylpseudoephedrine	179.3	29-31	+48	51018-28-1	

Appendix 3 Reported Ephedra plant species and compositions of Ephedra alkaloids

Table 5 Ratios of ephedra alkaloids in several Ephedra plant species [13,14]

Ephedra	Ratio EP/PE	Ratio ME/MPE	Ratio NE/NPE
E. sinica	> 1	> 10	> 0.4
E. intermedia	< 0.3	≈ 1	< 0.4
E. equisetina (Shennungiana)	> 1	≈ 10	≈ 0.4
E. distachya (Gerardiana)	> 1	≈ 5	< 0.4

EP ephedrine NE norephedrine PE pseudoephedrine
NPE norpseudoephedrine ME methylephedrine MPE methylpseudoephedrine

Table 6 Composition of Ephedra alkaloids in E. sinica and E. intermedia − commercially available extract (Netherlands)

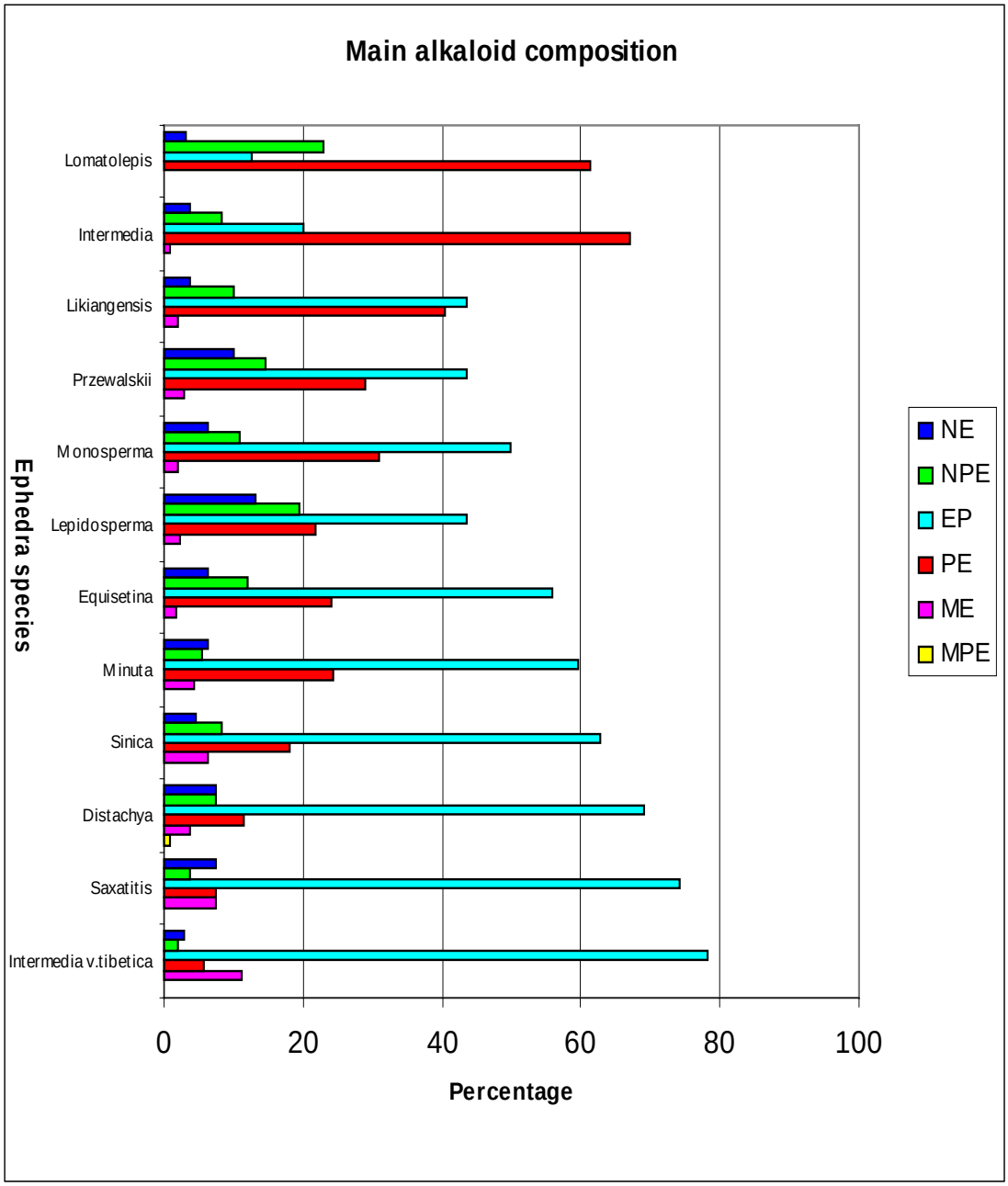
	EP (percent)	**PE** (percent)	**ME** (percent)	**MPE** (percent)	**NE** (percent)	**NPE** (percent)	***Ratio EP/PE***
E. Sinica [3] *E. Sinica* Range	57.5 38.4-78.2	29.7 8.6-49.2	6.4 4.1-9.0	0.7 0.4-1.0	2.5 1.3-3.6	3.5 1.4-5.4	*1.9* *1.6-4.5*
E. Herba, Dutch market '95	57.5-59.1	25.6-29.7	6.4-7.9	-	2.5-3.7	3.5-3.7	*1.9-2.3*
E. Intermedia [3] *E. Intermedia* Range	14.5 9.7-21.1	73.8 66.8-77.7	2.2 1.4-3.2	1.8 1.0-2.6	1.8 1.3-2.4	5.9 3.3-7.2	*0.2* *0.1-0.3*

EP ephedrine NE norephedrine PE pseudoephedrine
NPE norpseudoephedrine ME methylephedrine MPE methylpseudoephedrine

Table 7 Reported quality specifications for Ephedra herba in pharmacopeias

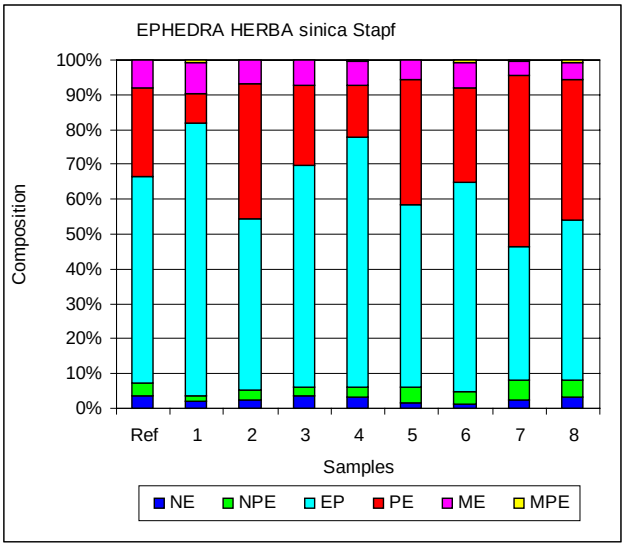
Substance	Appearance	Identification	Assay	Impurities	Pharmacopoeia
Ephedra herba	Dried stems or arial part of ephedrine alkaloid containing Ephedra genus	TLC with ninhydrine spray	0.7% or more total alkaloids as EP and PE	Acid-insoluble ash, maximum 2.0%, total ash maximum 11.0%.	Japanese Pharmacopoeia
Ephedra herba	Dried stems of ephedrine alkoloid containing Ephedra genus	TLC with ninhydrine	1.0% or more total alkaloids as EP	Ash maximum 9.0%, LOD maximum 9.0%, unusual substances maximum. 3%	Deutsche Arzneibuch
Ephedra herba	Dried stems or arial part of ephedrine alkaloid containing Ephedra genus	Microscopic	Refer to Chinese and Japanese Pharmacopoeias	Tests on microbiological purity, total ash, pesticide residue., Ash and heavy metalls, radioactive residue	International Pharmacopoeia
Ephedra herba	Dried stems or arial part of ephedrine alkaloid containing Ephedra genus	?	0.8% or more as EP	?	Chinese Pharmacopoeia

TLC Thin-layer chromatography LOD Loss on drying

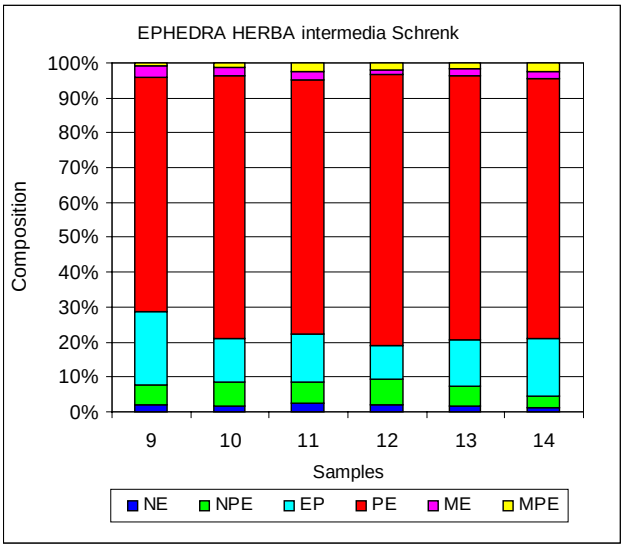


EP ephedrine NE norephedrine PE pseudoephedrine
NPE norpseudoephedrine ME methylephedrine MPE methylpseudoephedrine

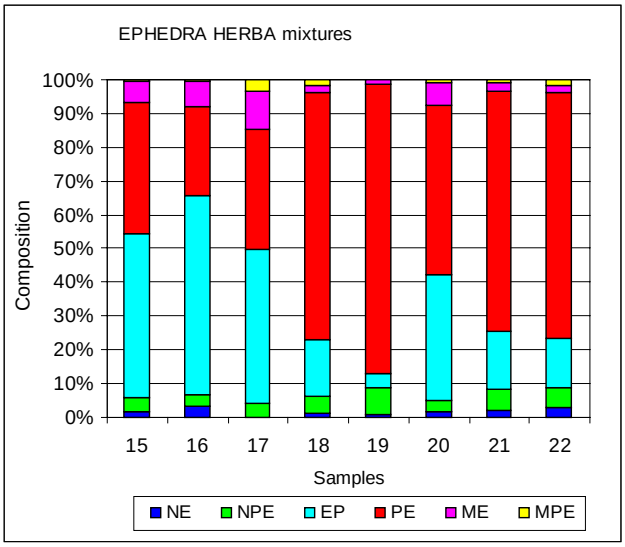
Figure 3 Ephedra alkaloid levels in Ephedra plant species [12]



a



b

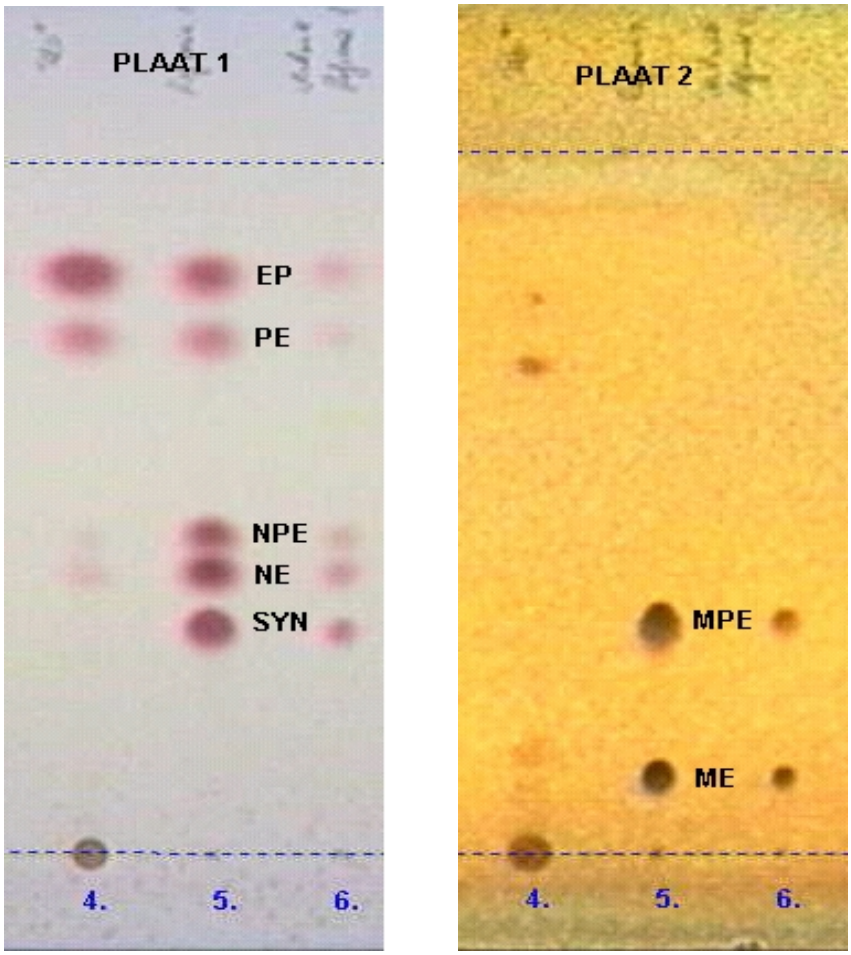


c

EP	ephedrine	NE	norephedrine	PE	pseudoephedrine
NPE	norpseudoephedrine	ME	methylephedrine	MPE	methylpseudoephedrine

Figure 4 a-c Composition of samples Ephedra herba available on the Taiwanese market [14]

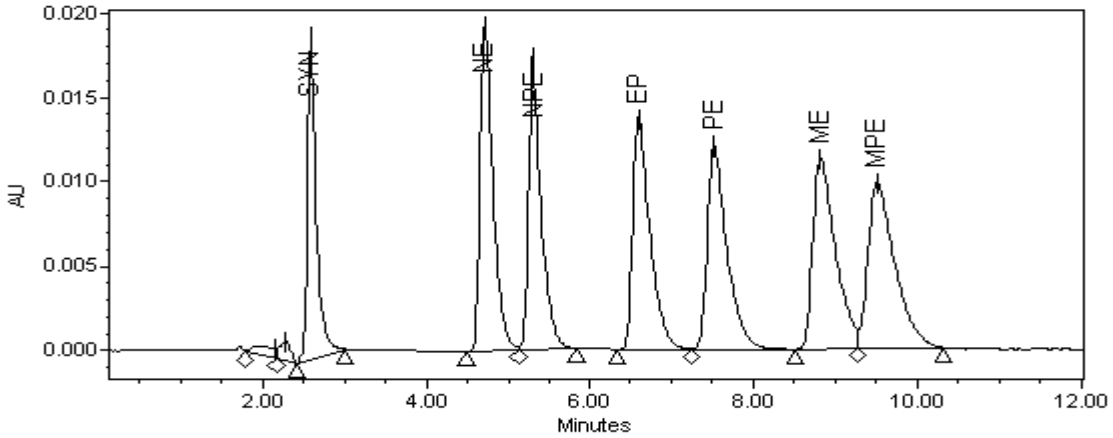
Appendix 4 Chromatograms



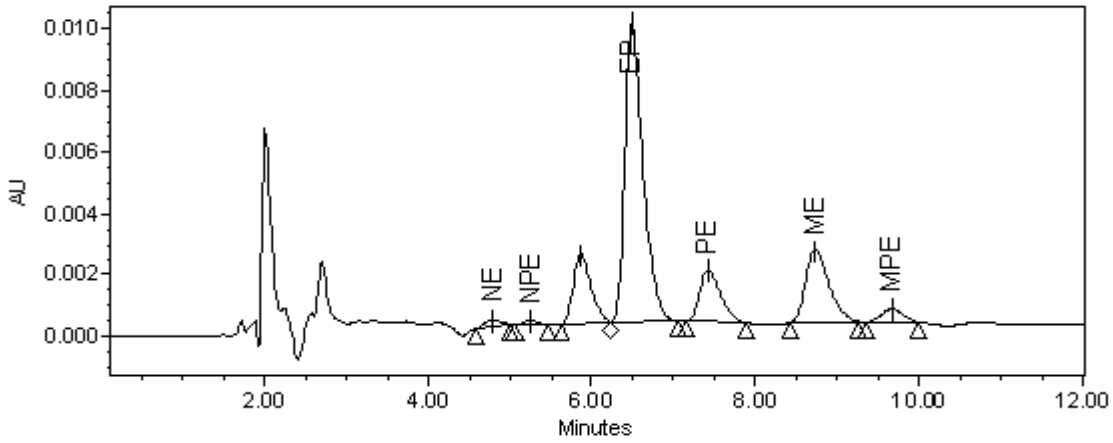
4.= Sample
5.= Reference mixture
6.= Diluted reference mixture

EP	ephedrine	NE	norephedrine	PE	pseudoephedrine
NPE	norpseudoephedrine	ME	methylephedrine	MPE	methylpseudoephedrine
SYN	synephrine				

Figure 5 Representative thin-layer chromatograms of sample and reference solutions in the identification tests



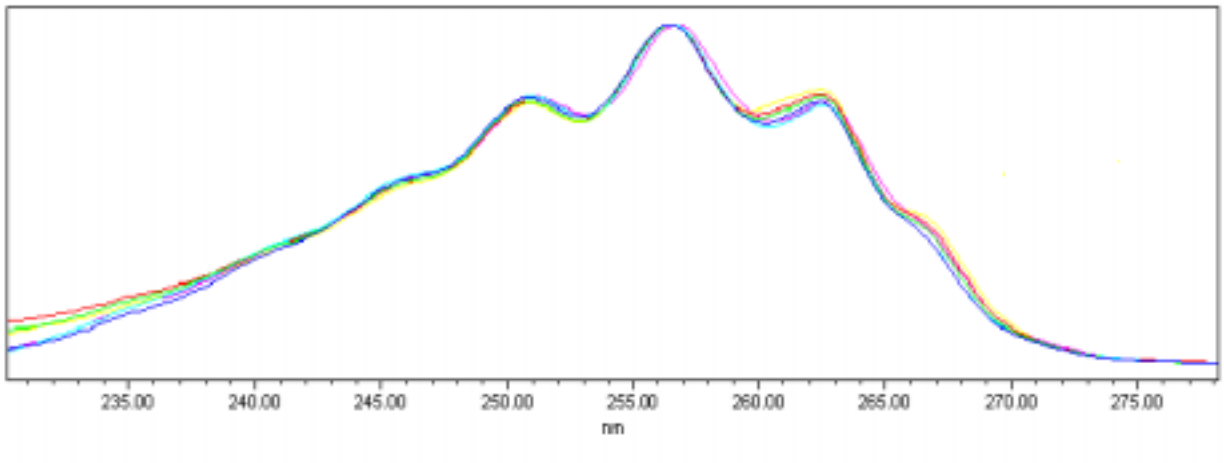
a System suitability solution



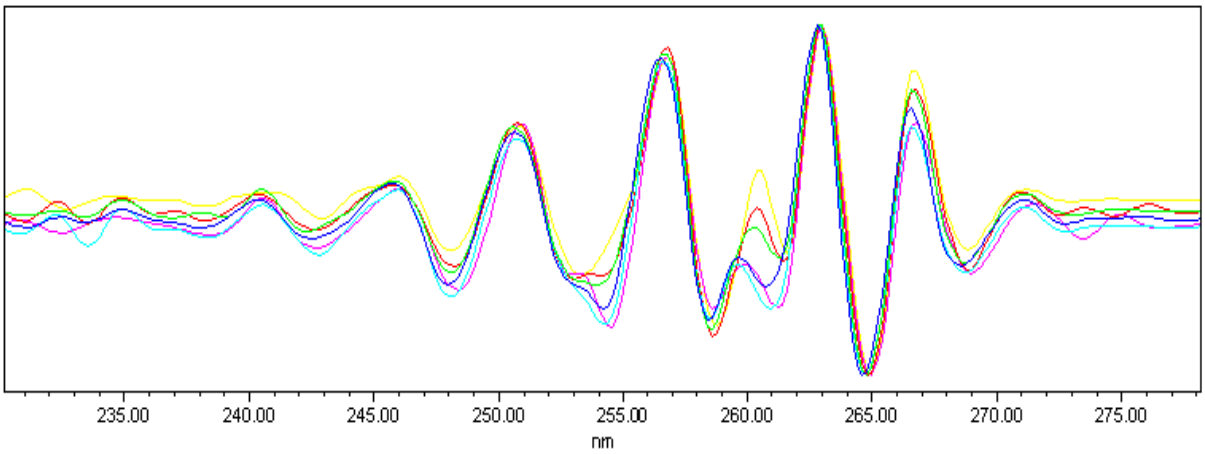
b Sample solution

EP	ephedrine	NE	norephedrine	PE	pseudoephedrine
NPE	norpseudoephedrine	ME	methylephedrine	MPE	methylpseudoephedrine
SYN	synephrine				

Figure 6 a,b Representative HPLC chromatograms of sample (b) and reference (a) solutions in the identification and assay tests (method: HPLC 2)



a DAD spectra



b Fourth derivative spectra

norephedrine
norspeudoephedrine
ephedrine
pseudoephedrine
methylephedrine
methylpseudoephedrine

Figure 7 a,b DAD-spectra and fourth derivative spectra of the peaks in a system suitability chromatogram (method: HPLC 2)

Appendix 5 Description of samples

Table 8a Dosage forms of the samples and the labelled active ingredients

Sample nr	Dosage form	Ephedra extract	Herbal ingredients	Other active ingredients
2492	Capsules	*Ephedra sinica* extract 833 mg	-	-
2493	Capsules	Indonesian MH 9% standardised 650 mg	-	-
2844	Tincture	MH extract	-	-
2864	Capsules	MH extract 500 mg	-	-
2865	Capsules	-	D, F, Go, GK, Gs	-
2866	Capsules	MH	Gu, GT, Go, KN, GK, WW	-
3122	Tablets		Gu, Gs, GK	BP, I, N
3123	Sachets	MH	-	b.Caro, Chr.asp., Cugl, Gly, Tau, Vit.C, E, A, Zngl
3125	Capsules	MH 300 mg	-	-
4015	Capsules	Indonesian MH 9% standardised 675 mg	-	-
4016	Capsules	Indonesian MH 9% standardised 650 mg	Gu, Gs, PF	-
4052	Capsules	MH 6% standardised 759 mg	-	N
4105	Tablets	EP (MH) 10 mg	Gu, St.JW	Ac.Car, Chr.asp., dCaP, N, Tau,Tyr, Vit. A, B3, B6, C, E
4106	Herb	*Ephedra nevadensis*	-	-
4107	Capsules	MH	F, Gu, Gi, Go, Hw, RH, WW, Yc	-
4108	Capsules	Ephedra 7% standardised	-	Zn
4109	Capsules	Zeedruif 850 mg	-	-
4110	Tablets	Ephedra	Gs, Gu, GK, PF, WB	-
4111	Tablets	MH	Av, Da, GK, Gs, Gi, Gr, Hw, Ra, RH, Sa, V, Ve, WB	FA, I, Mgch, PABA, Vit. C, E, A, B1, B2, B3, B5, B6, B12, Znch
4112	Capsules	*Ephedra sinica* extract 833 mg	-	-
4113	Tablets	Ephedra	Gu, Gs, GK, KN, PF, WB	KCl
4114	Tablets	MH	Gu, Gs, GK	BP, I, N
4115	Capsules	Ephedra 7% standardised	-	Zn
4116	Chewing gum	MH	Ca, Fu, Ga, Gu, KN, L, MS, Pa, WW	b.Caro, H, Znch
4117	Tablets	*Ephedra sinica* extract	Gu	-
4118	Capsules	Indonesian MH 9% standardised 675 mg	-	-
4119	Capsules	MH 8% standardised 750 mg	-	N
4120	Tablets	Tibetan MH	Alg, BP, F, Fu, Gi, Gu, Gs, L, WMB	-
4121	Capsules	MH 9% standardised 400 mg	Euphoric herb mix	-
4122	Capsules	Indonesian MH 9% standardised	-	-
4123	Capsules	MH extract 500 mg	-	-
4124	Tablets	*Ephedra Sinica* 125 mg	-	-
4125	Capsules	MH 6% 340 mg	Bu, Cay, Jb, KN, UU, WW	Car, Kglu, Chr.p, Vit. B6
4126	Capsules	MH 8% 253 mg	As, Cc, Gu, Cay, Bu, Jb, F, GC, Gs, GK, L, Me, P, Fe, KN, UU, WY, WW, Ye	Car, Chr.p. Kglu, Vit. B6
4127	Tablets	Ephedrine.HCl 20 mg	-	A, Br, C
4128	Capsules	MH 6% standardised 334 mg	Cay, Gu, WW	Chr.p
4201	Capsules	MH standardised 334 mg (~20 mg E.alk.)	Cay, Gu, WW	Chr.p
4328	Capsules	Ephedra extract	-	-
4329	Capsules	Ephedra	BP, Gs, KN, PF, RJ, Y	-
4330	Capsules	Ephedra extract	-	-
4331	Capsules	Ephedra extract	Gs, KN, WW	Chr
4332	Capsules	Ephedra	D, Gs, Gu, KN, PF, RJ	-
4333	Capsules	Ephedra extract	Gp, KN, WW	Ct
4338	Capsules	*Sida cordifolia* 10% standardised 500 mg	SpA	Glu, Pglu, PCh, Tyr, Vit.B6
4339	Capsules	*Sida acuta* 10% standardised 350 mg	ME, RC	Pa, Pglu,Tyr
4340	Capsules	MH 9% standardised 400 mg	Euphoric herb mix	-
4341	Capsules	MH 9% standardised 740 mg	-	-
4342	Capsules	*Epheda sinica* 8% standardised	-	-
4343	Tablets	MH 100 mg	Gu,Gs,GK	BP, I, N
4344	Capsules	*Ephedra sinica* extract 833 mg	-	-
4345	Tablets	Ephedra extract-333 mg	Gu	-
4346	Chewing gum	Ephedra	Gu, KN	-
4347	Capsules	MH	GK, Go, GT, Gu, KN, WW	-
4348	Herb	-	-	-
4349	Capsules	-	-	-
4350	Herb	-	-	-
4351	Powder	MH 1.67 g	-	Cu, Gly, Tau, Vit. A, C, E, Zn
4352	Sachets	Ephedra	Fe, Gi	-
4353	Capsules	MH	-	-

For description of abbreviations see Table 8b

Table 8b Description of abbreviations

Herbal ingredients

Alg	Pacific blue green algae
As	Astragalus *(A. membranaceus)*
Av	Oats *(Avena sativa)*
Bu	Buchu leaves *(Agathosma betulina)*
Cay	Cayenne *(Capsicum cayenne)*
Ca	Red pepper *(Capsicum annum)*
Cc	Caraway seed *(Carum carvi)*
D	Damiana *(Turnera diffusa)*
Da	Dandelion *(Taraxacum officinale)*
F	Foti-ti *(Polygonum multiflorum)*
Fe	Fennel seed *(Foeniculum vulgare)*
Fu	Sea kelp *(Fucus vesicosis)*
G	Gentian
Ga	Wintergreen *(Gaultheria procumbens)*
GC	Gardinia canbogia
Gi	Ginger *(Zingiber officinale)*
GK	Gotu Kola *(Hydrocotyle asiatica)*
Go	Ginko *(Ginko Biloba)*
Gp	Grapefruit extract
Gr	Garlic *(Allium sativa)*
Gs	Ginseng *(Panax ginseng)*
GT	Green tea
Gu	Guarana *(Paulina cupana)*
Hw	Hawthorne berry *(Crataegus fructus)*
Jb	Juniper berries *(Juniperi fructus)*
KN	Kola nut *(Cola nitida)*
L	Licorice *(Liquiritiae Radix)*
ME	*Maytenus Ehrifolia*
Me	Melissa *(Melissa officinalis)*
MS	Meadowsweet *(Filipendula ulmaria)*
P	Peppermint leaf *(Mentha piperitae folium)*
Pa	Paraquay tea *(Ilex paraguariensis)*
PF	Passion flower *(Passiflora herba)*
Ra	Raspberry *(Rubus spp)*
RC	Ololiuqui *(Rivea Corymbosa =Ipomea tricolor)*
RH	Rose hips
Sa	Savory *(Satureja hortensis)*
SpA	Spirulina microalgae
St.JW	St.John's Wort *(Hypericum)*
UU	Bearberry *(Uva Ursi)*
V	Valerian *(Valeriana officinalis)*
Ve	Verbena *(Verbena officinalis)*
WB	Wood betony *(Stachys officinalis)*
WMB	Wild mushroom blend
WW	White willow bark *(Salix alba)*
WY	Mexican wild yam *(Dioscorea villosa)*
Y	Yohimbe *(Pausinystalia yohimbe)*
Yc	Yucca
Ye	Yerba mate *(Eriodictyon californicum)*

Ephedra extract

MH	Ma Huang
Std	Standardised extract

Other active ingredients

A	Aspirin
Ac.Car	Acetyl-L-carnitine
b.Caro	beta-Carotene
Bi	Biotin (vit. B7)
BP	Bee pollen
Br	Bromelase
C	Caffeine
Car	Carnitine
Ch	Chromium
Chr.asp	Chromium aspartate
Chr.p	Chromium picolinate
Ct	Chitosan
Cu	Copper
Cugl	Copper gluconate
dCaP	Di calcium phosphate
FA	Folic acid (vitamin B11)
Glu	Glutamine
Gly	Glycine
H	Histidine
I	Inosine
KCl	Potassium chloride
Kglu	Potassium gluconate
Mgch	Magnesium chelate
N	Niacine
Pa	Phenylalanine
PABA	Para-aminobenzoic acid
Pch	Phosphatidylcholine
Pglu	Pyroglutamine
RJ	Royal jelly
Tau	Taurine
Tyr	l-Tyrosine
Vit. A	Retinol
Vit. B1	Thiamine
Vit. B2	Riboflavine
Vit. B3	Nicotinamide
Vit. B5	Pantothenic acid
Vit. B6	Pyridoxine
Vit. C	Ascorbic acid
Vit. E	Tocopherol
Zn	Zinc
Znch	Zinc chelate
Zngl	Zinc gluconate

Appendix 6 Quality results (studies 1, 2, 3) and safety results (studies 2,3)

Table 9 Quality results of study 1: composition of ephedrine alkaloids in the samples that were not from natural source

Sample*	Lot no.	NPE (as HCl)	EP (as HCL)	Other**	Percent ***	Natural source****
A1	VB15I3	7.7 mg/ml	--	--	100	-
A2	VB25J94	16 mg/ml	--	--	100	-
A3	VB15B95	13.6mg/ml	--	--	100	-
A4	VB08F95	10.2 ± 0.5mg/ml (n = 2)	--	--	100	-
A5	VB01G95	6.5 ± 0.3mg/ml (n = 5)	--	--	100	-
A6	VB05G4	6.7 mg/ml	--	--	100	-
A7	VB01G25	6.5 mg/ml	--	--	100	-
A8	VB27G95	7.7 +1.9/-0.9(n = 3)	--	--	100	-
A9#	DH16G95	0.01	--	--	Trace	Not assigned
A10#	DH01B96	--	--	--	0	+
B1	-	8.8 mg/ml	--	--	100	-
B2	-	8.9 mg/ml	--	--	100	-
B3	-	8.3 mg/ml	--	--	100	-
B4	-	10.4 mg/ml	--	--	100	-
B5	-	10.6 mg/ml	--	--	100	-
B6	-	15.6 mg/ml	--	--	100	-
B7	-	6.4 mg/ml	--	--	100	-
B8	-	4.6 mg/ml	--	--	100	-
B9#	-	--	--	--	0%	+
C1	-	14 mg/ml	--	--	100	-
C2	-	--	--	--	100	-
C3	-	--	--	--	100	-
D	L649/173	10 mg/capsule	0.3 mg/capsule	PE 0.3 mg/capsule	94.3 2.8 2.8	-[2]
D	-	11.8 mg/ml	--	NE 6.7 mg/ml	63.8 36.2	-[2]
E	54896		4.1 mg/tablet		100	-
F1	-	--	3.5 mg/ml	PE 2.1 mg/ml ME 0.5 mg/ml	57.4 34.4 8.2	-[1]
F2	-	--	3.5 mg/ml	PE 2.1 mg/ml ME 0.2 mg/ml	54.4 41.3 43.5	-[1]

* Sample 'A1' means: product A, sample no. 1, etc.
** Other naturally occuring ephedrine alkaloids detected besides EP and NPE, e.g. NE, ME, PE
*** Percentage found in relation to total ephedrine alkaloids present in the product
**** Evaluation whether from natural source:
+ probable
± possible
-[1] probable, but enriched with synthetic ephedrine
-[2] probable, but enriched with synthetic ephedrine alkaloids
- unlikely
-- Not detected
New formula (NF) introduced after action of Dutch Inspectorate

Table 10 Quality results of study 2: composition of the ephedrine alkaloids and natural origin classification

Sample no.	Total alkaloids (mg/g)	EP* (Percent)	PE* (Percent)	ME* (Percent)	NE* (Percent)	NPE* (Percent)	EP/PE Ratio	Natural source**
2492	18.9	77.1	15.3	3.7	3.1	0.8	5.0	+
2493	73.3	97.1	2.2	--	0.6	0.1	44.1	-[1]
2844	21 mg/ml	9.5	76.2	14.3	Traces	Traces	0.1	+
2864	43.9	75.4	22.0	2.6	--	--	3.4	+
2865	12.4	13.4	70.2	--	--	--	0.2	±
2866	12.7	14.9	70.2	--	--	--	0.2	±
3122	--	--	--	--	--	--	Not relevant	Not assigned
3123	0.1	100.0	--	--	--	--	Not relevant	±
3124	--	--	--	--	--	--	Not relevant	Not assigned
3125	4.1	57.6	29.0	7.0	0.8	5.6	2.0	+
3999	41.7	61.1	31.7	1.8	--	5.5	1.9	+
4014	35.1	63.8	33.9	0.1	0.1	2.2	1.9	+
4015	54.7	95.9	3.8	--	--	0.3	25.2	-[1]
4016	29.5	83.7	11.3	5.0	--	--	7.4	+
4052	76.3	80.9	18.4	--	0.1	0.6	4.4	+
4105	9.6	69.8	30.2	--	--	--	2.3	±
4106	12.8	61.2	34.0	4.8	Traces	Traces	1.8	+
4107	16.8	78.3	21,7	--	--	--	3.6	±
4108	68.4	44.1	50.8	5.2	Traces	Traces	0.9	+
4109	12.7	63.8	36.2	--	--	--	1.8	±
4110	13.7	76.2	23.8	--	Traces	Traces	3.2	+
4111	1.7	74.9	25.1	--	--	--	3.0	±
4112	51.4	100.0	--	--	--	--	Not relevant	-
4113	13.1	76.7	23.3	--	Traces	Traces	3.3	+
4114	4.9	67.3	32.7	Traces	--	--	2.1	+
4115	66.5	66.1	14.7	19.2	--	Traces	4.5	+
4116	0.2	100.0	--	--	--	--	Not relevant	±
4117	26.3	22.0	78.0	--	Traces	Traces	0.3	+
4118	87.4	95.3	4.7	--	Traces	Traces	20.2	-[1]
4119	66.8	60.7	31.0	2.7	Traces	5.7	2.0	+
4120	7.5	100.0	--	--	--	--	Not relevant	-
4121	34.9	38.8	61.7	Traces	Traces	Traces	0.6	+
4122	54.1	75.4	24.6	--	--	Traces	3.1	+
4123	61.5	56.6	43.4	--	Traces	Traces	1.3	+
4124	7.4	65.8	27.7	Traces	--	Traces	2.6	+
4125	11.6	52.9	47.1	--	--	Traces	1.1	+
4126	12.5	34.3	65.7	Traces	--	Traces	0.5	+
4127	42.9	100.0	--	--	--	--	Not relevant	-
4128	14.7	9.1	70.6	20.3	--	Traces	0.1	+
4201	14.4	78.7	15.1	6.1	Traces	Traces	5.1	+
4328	55.5	89.9	8.1	Traces	--	Traces	11.1	+
4329	41.2	77.6	22.5	--	--	--	3.5	±
4330	62.5	86.8	8.9	4.3	--	Traces	9.8	+
4331	41.2	78.9	19.1	Traces	--	Traces	4.1	+
4332	30.5	75.3	23.0	Traces	--	Traces	3.3	+
4333	40.3	75.3	23.0	Traces	--	Traces	3.3	+
5019	46.0	56.7	37.5	--	<2.9	<2.9	1.5	+
5020	47.4	58.6	35.2	--	<3.1	<3.1	1.7	+

* Percentage found in relation to total ephedrine alkaloids present in the product.

** Evaluation whether from natural source:

- \+ probable
- ± possible
- -[1] probable, but enriched with synthetic ephedrine
- -[2] probable, but enriched with other synthetic ephedrine alkaoids
- \- unlikely

-- Not detected

Table 11 Quality results of study 3: Composition of ephedrine alkaloids and natural origin classification

Sample no.	Total alkaloids (mg/g)	EP* (percent)	PE* (percent)	ME*^ (percent)	MPE*^ (percent)	NE* (percent)	NPE* (percent)	EP/PE Ratio	Natural source**
4338	51.5	89.3	10.7	Trace	^	--	Trace	8.3	+
4339	83.1	100.0	--	--	^	--	--	--	-
4340	46.1	82.2	17.8	Trace	^	--	Trace	4.6	+
4341	95.7	82.7	12.9	4.5	^	Trace	Trace	6.4	+
4342	67.1	86.7	13.3	Trace	^	--	Trace	6.5	+
4343	3.4	100.0	Trace	--	^	--	--	2.5	±
4344	51.3	100.0	--	--	^	--	--	Not relevant	-
4345	13.5	100.0	--	--	^	--	--	Not relevant	-
4346	0.2	100.0	Trace	--	^	--	--	2.4	±
4347	< 1	--	--	--	^	--	--	Not relevant	Not assigned.
4348	10.2	72.4	27.6	Trace	^	Trace	Trace	2.6	+
4349	9.4	70.8	23.8	4.1	^	Trace	5.5	3.0	+
4350	13.1	70.0	17.0	Trace	^	Trace	9.4	4.1	+
4351	0.8	100.0	Trace	--	^	--	--	7.7	±
4352	1.0	100.0	Trace	--	^	--	Trace	3.0	±
4353	<0.8	--	--	--	^	--	--	Not relevant	Not assigned.
4637	69.5	64.8	11.6	16.7	--	--	< 7.0	5.6	+
4638	< 0.4	--	--	--	--	--	--	Not relevant	Not assigned.
4639	< 0.3	--	--	--	--	--	--	Not relevant	Not assigned.
4640	< 0.5	--	--	--	--	--	--	Not relevant	Not assigned.
4641	13.1	85.4	7.5	< 7.2	--	--	--	11.5	+
4642	28.4	45.1	46.8	< 4.1	--	--	< 4.1	1.0	+
4643	33.8	87.4	< 6.3	--	--	--	< 6.3	13.8	+
4644	37.0	74.3	14.1	< 5.8	--	--	< 5.8	5.3	+
4645	44.7	58.3	35.1	< 6.6	--	--	-	1.7	+
4646	69.9	64.0	12.1	17.2	--	--	< 6.7	5.3	+
4647	7.7	76.3	23.7	--	--	--	-	3.2	±
4648	31.5	45.8	47.2	< 3.5	--	--	< 3.5	1.0	+
4649	62.3	100.0	--	--	--	--	--	Not relevant	-
4650	43.7	57.3	33.1	--	--	< 4.8	< 4.8	1.7	+
4651	< 0.3	--	--	--	--	--	--	Not relevant	Not assigned.
4652	66.7	65.0	12.1	17.5	--	--	< 5.4	5.4	+
4653	69.7	100.0	--	--	--	--	--	Not relevant	-
4690	65.7	66.8	11.9	17.0	--	--	< 4.3	5.6	+
4691A***	41.1	22.4	41.7	29.9	--	< 3.0	< 3.0	0.5	+
4691B	53.2	22.8	42.1	30.8	< 4.3	--	--	0.5	+
4692	10.1	68.1	26.6	--	--	--	< 5.3	2.6	+
4693	Not relevant	91.3	8.7	--	--	--	--	10.5	-
4694	Not relevant	35.1	56.2	< 4.3	--	--	< 4.3	0.6	+
4695	7.0	100.0	--	--	--	--	--	Not relevant	-
4696A***	68.1	40.8	41.8	< 5.8	--	< 5.8	< 5.8	1.0	+
4696B	74.8	42.0	42.4	< 5.2	--	< 5.2	< 5.2	1.0	+
4697	84.2	52.8	26.6	< 5.2	< 5.2	< 5.2	< 5.2	2.0	+
4698	44.3	33.2	52.3	< 4.8	-	< 4.8	< 4.8	0.6	+
4699	21.0	20.4	56.0	16.2	< 3.7	--	< 3.7	0.4	+
4700	36.3	100.0	--	--	--	--	--	Not relevant	-
4701	67.1	61.1	27.0	--	--	< 5.9	< 5.9	2.3	+
4702	< 0.4	--	--	--	--	--	--	Not relevant	Not assigned.
4703	5.0	72.5	27.5	--	--	--	--	2.6	±
4704	< 0.3	--	--	--	--	--	--	Not relevant	Not assigned.
4705	2.7	100.0	--	--	--	--	--	Not relevant	-
4706	< 4.6	< 50	< 50	--	--	--	--	Not relevant	±
4707	67.4	69.6	24.0	--	--	--	< 6.4	Not relevant	+
4708	27.5	20.5	73.5	--	< 2.9	--	3.0	0.3	+
4709	38.5	26.0	43.3	30.7	--	--	--	0.6	+
4710	20.5	65.2	27.8	< 7.0	--	--	--	2.3	+
4711	67.9	63.5	12.3	17.6	--	--	< 6.6	5.2	+
4712	14.5	60.9	31.5	--	--	--	7.6	1.9	+
4713	0.7	65.2	34.8	--	--	--	--	1.9	±
4714	< 0.6	--	--	--	--	--	--	Not relevant	Not assigned.
4715	8.9	70.1	18.1	< 5.6	--	--	< 5.9	3.9	+
4716	1.0	75.5	24.5	--	--	--	--	3.1	±
4717	6.0	84.9	15.1	--	--	--	--	5.6	±

4718	68.5	54.0	29.1	< 5.6	--	< 5.6	< 5.6	1.9	+
4719	1.0	100.0	--	--	--	--	--	Not relevant	-
4720	53.8	72.1	13.5	< 7.2	--	--	< 7.2	5.4	+
4721	48.2	76.0	14.8	< 4.6	--	--	< 4.6	5.1	+
4722	46.1	71.0	14.4	< 7.3	--	--	< 7.3	4.9	+
4723	13.5	72.0	< 10.3	--	--	--	< 10.3	7.7	±
4724	14.1	79.9	13.3	< 6.7	--	--	--	6.0	+
4725	7.9	70.5	29.5	--	--	--	--	2.2	±
4726	92.3	88.7	< 3.8	< 3.8	--	--	< 3.8	23.5	-
4727	17.3	78.9	13.3	< 7.9	--	--	--	5.9	+

* Percentage found in relation to total ephedrine alkaloids present in the product

^ The samples up to sample no. 4637 were determined with HPLC1 so for these samples ME and MPE were not separated; the samples starting from no. 4637 were determined with HPLC2, with separation between ME and MPE.

** Evaluation whether from natural source:

+ probable

± possible

-[1] probable, but enriched with synthetic ephedrine

-[2] probable, but enriched with other synthetic ephedrine alkaoids

- unlikely

-- Not detected, level below detection limit (0.005mg/ml)

< Detected, level below quantification limit (0.01 mg/ml)

*** The sample contained two types of capsules (different appearances); these were analysed seperately

Table 12 Safety results of study 2: total content of ephedrine alkaloids, classification by FDA safety ranges, and xanthine derivative labelling

Sample no.	Total alkaloids (mg/dose unit)	Labelled recommended dose units	maximum daily dose (mg)	FDA* compliance	Xanthine derivate labelled
2492	13.2	1 capsule/day	13	-	
2493	52.8	1-2 capsules	106	-	
2844	21 mg/ml	30-40 drops (1.5-2ml)	42	-	
2864	23.2	maximum 3 capsules/day	70	-	
2865	6.7	?	?	-	Yes
2866	6.7	3-5 capsules	34	+	Yes
3122	< 0.5	2 capsules, maximum 6 capsules/day	< 3	+	Yes
3123	1.5	1-3 sachets/day	5	+	
3124	<1.3 mg/caps	5 capsules	< 6.5	+	
3125	4.1	maximum 8 capsules/day	33	+	
3999	39.0	maximum 3 capsules/day	117	-	
4014	32.3	maximum 3 capsules/day	97	-	
4015	40.9	1-2 capsules	82	-	
4016	29.5	1-4 capsules	118	-	Yes
4052	70.3	maximum 3 capsules/day	210	-	
4105	10.5	2 capsules, maximum 4 capsules/day	42	-	Yes
4106	13 /g	?	?	-	
4107	10.9	3 capsules	33	-	Yes
4108	67.6	Female: 1 capsule, male: 2 capsules maximum 3 capsules/24 hours	203	-	
4109	3.8	2 capsules	8	+	
4110	16.3	3 capsules	49	-	Yes
4111	1.7	1 – 2 tablets	3	+	Yes
4112	42.0	1 capsules/day	42	-	
4113	16.5	1 tablet, maximum 3 tablets/6 hours	198	-	Yes
4114	7.6	2 tablets, maximum 6 tablets/day	46	-	Yes
4115	61.1	2 capsules	122	-	
4116	0.2 mg/gum	?	?	-	
4117	19.4	1 tablet/72 hours	19	-	Yes
4118	61.7	1-2 capsules	124	-	
4119	62.2	maximum 3 capsules/day	187	-	
4120	6.1	3 – 5 tablets	31	+	Yes
4121	19.6	3 capsules, maximum 3 capsules/24 hours	59	-	
4122	41.4	1 capsule/72 hours	41	-	
4123	33.1	3 capsules, maximum 3 capsules/day	99	-	
4124	5.5	1 tablet/day	6	+	
4125	8.6	2 capsules, maximum 6 capsules/day	52	-	Yes
4126	10.7	2 capsules	21	+	Yes
4127	29.8	2x1 tablet or 3x1 tablet/day	89	-	
4128	11.0	2 capsules, maximum 6 capsules/day	66	-	Yes
4201	10.7	2 capsules, maximum 6 capsules/day	64	-	Yes
4328	28.9	1 capsule, maximum 3 capsules/day	87	-	
4329	21.8	1 capsule, maximum. 3 capsules/day	65	-	
4330	25.9	1 capsule, maximum 3 capsules/day	78	-	
4331	21.6	1 capsule, maximum 3 capsules/day	65	-	
4332	15.9	1 capsule, maximum 3 capsules/day	48	-	
4333	21.1	1 capsule, maximum 3 capsules/day	63	-	
5019	29.9	4 capsules/day	119.6	-	Yes
5020	31.8	4 capsules/8 hours	127.2	-	Yes

* + In conformity with FDA limits
- Not in conformity with FDA limits or a dose indication is lacking

? A dose indication is lacking

Table 13 Safety results of study 3: Total content of ephedrine alkaloids, classification by FDA safety ranges, and labelled Xanthine derivatives

Sample nr	Total alkaloids (mg/dose unit)	Labelled recommended dose units	Maximum daily dose (mg)	FDA compliance*	Xanthine derivate labelled
4338	19.4	1-2 capsules, maximum 4 capsules/day	78	-	
4339	31.0	1-2 capsules, maximum 4 capsules/day	124	-	
4340	22.0	3 capsules, maximum 3 capsules/day	66	-	
4341	55.2	3 capsules, maximum 3 capsules/day	166	-	
4342	38.3	1-2 capsules, maximum 4 capsules/day	153	-	
4343	3.5	2 tablets	7	+	
4344	37.0	1 capsules/day	37	-	
4345	23.0	1-2 tablets/day	46	-	
4346	0.2	?	0.2	+	
4347	<1 mg/g	3-5 capsules	Not relevant	+	
4348	10.2 mg/g	?	?	-	
4349	2.2	?	?	-	
4350	13.1 mg/g	?	?	-	
4351	0.8 mg/g	1 table spoon	?	+	
4352	1.9	?	?	-	
4353	<0.8 mg/g	?	Not relevant	+	
4637	60.4	1 – 2 capsules	120.8	-	
4638	< 0.2	1 – 4 capsules/day	< 0.8	+	
4639	< 0.3	4 tablets/day	< 1.2	+	
4640	< 0.3	?	Not relevant	+	
4641	15.0	4 tablets/day	60	-	Yes
4642	15.9	2 capsules/day	31.8	-	
4643	18.6	1 – 4 capsules	74.4	-	Yes
4644	19.8	1 – 4 capsules	79.2	-	Yes
4645	27.9	4 capsules/8 hours	334.8	-	Yes
4646	59.0	3 capsules/day	177	-	
4647	5.9	2 capsules/day	11.8	+	Yes
4648	17.6	4 capsules/8 hours	211.2	-	Yes
4649	51.8	4 capsules/day	207.2	-	
4650	29.6	2 capsules	59.2	-	
4651	< 0.2	5 tablets/day	< 1	+	
4652	58.9	2 capsules	117.8	-	
4653	65.8	6 tablets/4-5 hours	394.8	-	Yes
4690	69.0	3 capsules/day	207	-	
4691A***	30.3	?	?	-	
4691B	46.8			-	
4692	10.1mg/g	1 teaspoon (3 g)	46.8	-	
4693	8.8 mg/ml	30 – 40 drops (1.5-2 ml)	17.6	-	
4694	8.7 mg/ml	15 ml	130.5	-	
4695	5.6	3 – 5 tablets	28	-	Yes
4696A***	64.0	3 capsules/day	192	-	
4696B	73.7		221.1	-	
4697	75.5	3 capsules/day	226.5	-	
4698	25.3	3 capsules/day	75.9	-	
4699	15.5	1 tablet/12 hours	31	-	Yes
4700	20.9	4 capsules/day	83.6	-	
4701	42.2	4 capsules/day	168.8	-	
4702	< 0.2	?	Not relevant	+	
4703	2.2	2 – 4 capsules	8.8	+	Yes
4704	< 0.4	2 capsules	< 0.8	+	
4705	3.3	2 capsules	6.6	+	
4706	< 1.1	?	Not relevant	-	
4707	58.3	4 capsules/day	233.2	-	
4708	21.5	1 tablets/72 hours	7.2	-	
4709	30.1	2 capsules	60.2	-	
4710	22.2	2 tablets	44.4	-	
4711	58.7	1 – 2 capsules	117.4	-	
4712	1.5	8 capsules/day	12	+	
4713	0.8	?	?	-	Yes
4714	< 0.2	?	Not relevant	-	
4715	14.0	6 tablets/day	84	-	Yes
4716	1 mg/g	2 bottles/day	?	+	Yes
4717	8.9	6 tablets/day	53.4	-	
4718	52.5	4 capsules/day	210	-	
4719	1 mg/g	3 cups of tea/day.	?	+	
4720	31.9	3 capsules/day	95.7	-	Yes

4721	30.3	?	?	-	Yes
4722	30.2	3 capsules/day	90.6	-	Yes
4723	10.5	4 tablets/day	42	-	Yes
4724	10.1	2 – 3 tablets	30.3	-	Yes
4725	1.2	1 – 4 capsules	4.8	+	
4726	63.9	2 capsules	127.8	-	
4727	13.8	6 capsules/day	82.8	-	Yes

* + In conformity with FDA limits
- Not in conformity or a dose indication is lacking

? A dose indication is lacking

*** The sample contained two types of capsules (different appearance); these were analysed seperately

Appendix 7 Declaration of quality control

Undersigned states herewith that the research presented in this report has been carried out according to the OECD principles of Good Laboratory Practice (GLP) and that this report reflects a complete, correct and reliable overview of the results obtained.

GLP inspections of the experiments and reports submitted to the management research team leader took place on:

Date	Report publication date	Report inspection date
10-12-1996		10-12-1996
08-03-1999		08-03-1999
04-10-1999		04-10-1999

This report was inspected on 04-12-2000
Inspection of report no. 670220001

Quality control officer:

name : G.M. Overvliet
laboratory : LGO (RIVM)
date : 04-10-2000
signature :

Appendix 8 Mailing list

01. Prof. H.J. Schneider, Directeur-Generaal Volksgezondheid, VWS
02. Dr.ir. M.W.J. Wolfs, Algemeen Hoofdinspecteur, KvW, VWS
03. Drs. H. de Sitter, Inspecteur Food, KvW, VWS
04. Drs. B.M. Kustner, Inspecteur Food, KvW, VWS
05. Mr. L.J.S. Wever, wnd. Directeur, GMV, VWS
06. Drs. A.A.W. Kalis, Directeur, GZB, VWS
07. Ir. R. Top, GZB, VWS
08. Prof.dr. J.H. Kingma, Inspecteur-Generaal, HIGZ, VWS
09. Drs. P.H. Vree, plv. Inspecteur-Generaal voor de Gezondheidszorg, HIGZ, VWS
10. Drs. J.M.M. Hansen, wnd. Hoofdinspecteur voor de Farmacie en de Medische Technologie, HIGZ, VWS
11. Dr. C.A. Rutgers, HIGZ, VWS
12. Dr. R.J.J.Ch. Lousberg, Inspecteur Opiumwetzaken, HIGZ, VWS
13. Drs. M.G.A.M. Moester, Inspecteur farmacie, RIGZ, VWS
14. De heer A. van Nes, Adjunct-inspecteur, RIGZ, VWS
15. Mw. W. Verdonk-Kleinjan, KvW, VWS
16. Ir. H.M.M. Roomans, KvW, VWS
17. Prof.dr. J.J. Sixma, Voorzitter, Gezondheidsraad
18. Dr. H. Huizer, NFI
19. Prof.dr. A.W. Broekmans, Directeur, ACBG, VWS
20. dr. J.F.F. Lekkerkerker, Voorzitter, ACBG, VWS
21. Dr. W.G. van der Sluis, Universiteit Utrecht
22. Prof.dr. F.A. de Wolff, Universiteit Leiden
23. Prof.dr. A.J. Vlietinck, Universiteit van Antwerpen
24. NECEDO
25. Dr.ir. G. de Mik (Sector RMG)
26. Dr. P. van Zoonen (LOC)
27. Dr.ir. HJGM Derks (LGO)
28. Prof.dr. R.W. Stephany (ARO)
29. Dr. G. Zomer (LOC)
30. Dr.ir. E.H.J.M. Jansen (LEO)
31. Drs. S.S. Sterk (ARO)
32. Dr. L.A. van Ginkel (ARO)
33. Dr. H.P. van Egmond (ARO)
35. Dr. J. Meulenbelt (NVIC)
36. Prof. dr. P.W.J. Peters, KvW, VWS
37. Dr. P.P. Beljaars, KvW, VWS

38 - 39 Bibliotheek KvW

40 - 44. Auteurs

45 - 50. SBD/Voorlichting en Public Relations

51. Bureau Rapportenregistratie
52. Bibliotheek RIVM

53 - 63. Bureau Rapportenbeheer

64 - 80. Reserve exemplaren

81. Depot Nederlandse Publikaties en Nederlandse Bibliografie