Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1107 (2006) 9–18
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Solid–liquid extraction and cation-exchange solid-phase extraction using a mixed-mode polymeric sorbent of *Datura* and related alkaloids

Tomasz Mroczek *, Kazimierz Głowniak, Joanna Kowalska

Department of Pharmacognosy with Medicinal Plants Laboratory, Medical University, 1 Chodźki St., 20-093 Lublin, Poland

Received 24 October 2005; received in revised form 6 December 2005; accepted 7 December 2005
Available online 4 January 2006

Abstract

Tropane alkaloids solid–liquid extraction methods were developed and comprised ambient pressure ones: extraction with hot solvent, extraction at room temperature, on ultrasonic bath as well as pressurised liquid extraction (PLE) techniques. The highest yields of L-hyoscyamine in methanol PLE method (3 × 5 min, 110 °C) and scopolamine extracted with 1% tartaric acid in methanol (15 min, 90 °C) were determined. A mixed-mode reversed-phase cation-exchange solid-phase extraction (SPE) procedure was optimised for simultaneous recoveries of L-hyoscyamine, scopolamine, scopolamine–*N*-oxide from plant extracts as well as quaternary alkaloid representative: scopolamine–*N*-methyl bromide. First three alkaloids were efficiently eluted (recoveries 80–100%) from an Oasis MCX cartridge with methanol–10% ammonia (3:1, v/v) solution, whereas for the quaternary salt tetrahydrofuran–methanol–25% ammonia (6:1:3, v/v) was used with recoveries 52–6%. HPTLC-densitometric assay on silica gel plates was elaborated at 205 nm without derivatization and included: single development (over a distance 9.5 cm) with acetone–methanol–water–25% ammonia (85:5:5:8, v/v) mobile phase for L-hyoscyamine and scopolamine separation, whereas for scopolamine–N-oxide and scopolamine–*N*-methyl bromide a second development (to a distance 5.5 cm) with acetonitrile–methanol–85% formic acid (120:5:5, v/v) was applied. Newly elaborated RP-HPLC-diode array detection method was performed on Waters XTerra RP-18 column with gradient of acetonitrile in 15 mM ammonia solution and alkaloids were baseline separated within 20 min. Both chromatographic methods were validated and their quantitative results were compared. Good correlation between HPLC and HPTLC quantitative results was measured (correlation coefficients of mean values were 0.92086 and 0.99995 for L-hyoscyamine and scopolamine, respectively). In the RP-HPLC method, which was from 1.5- up to 7-fold more sensitive than HPTLC, limits of detection (LOD) and limits of quantitation (LOQ, in bracket) were (in ng/μl) as follows: 0.25 (0.82) for L-hyoscyamine, 0.29 (0.97) for scopolamine, 0.13 (0.45) for scopolamine–*N*-oxide and 0.58 (1.91) for scopolamine–*N*-methyl bromide. By the use of the optimised chromatographic methods, 14 various samples from the leaves and fruits of *Datura* sp. were screened for L-hyoscyamine and scopolamine contents and the most promising samples were established.

Keywords: Tropane alkaloids; Solid–liquid extraction; Solid-phase extraction; Chromatographic techniques

1. Introduction

Tropane alkaloids are derivatives of tropane, which chemically is 8-azabicyclo[3.2.1]octane. In typical classification two groups of tropane derivatives are considered: as tropine (*endo*-8-azabicyclo[3.2.1]octane-3-ol) and pseudotropine (*exo*-8-azabicyclo[3.2.1]octane-3-ol) alkaloids [1,2]. These two bases are esterified with various acids usually optically active, such as tropic acid, apotropic, cinnamic, angelic, tiglic, isovaleric and α-truxillic acids. Recently, a new class of *nor*-pseudotropine alkaloids called calystegines was found [4].

Long before the elucidation of their structures, the pharmacological properties of several tropane alkaloids were exploited [2]. Atropine, which typifies the action of tropane alkaloids, causes antagonism to muscarine receptors (parasympathetic inhibition) [2]. Cocaine is perhaps the best known of all tropane alkaloids mainly because of its use as an illicit drug [2]. The alkaloids present in thorn apple (*Datura stramonium* L.), mainly L-hyoscyamine and scopolamine, are included in many official Pharmacopoeias because of their anticholinergic activities [3].

* Corresponding author. Tel.: +48 81 7410351; fax: +48 81 7410351.
E-mail address: tmroczek@pharmacognosy.org (T. Mroczek).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.12.034

For the extraction of tropane alkaloids, the following solvents are described: diluted sulphuric(VI) acid [5], acetic acid [6], chloroform, dichloromethane, benzene or toluene in alkaline pH [7,8], ethanol–25% ammonia mixture [9], ethanol, methanol, methanol–water mixtures (for calystegines extraction) [10,11], supercritical carbon dioxide and their mixtures with methanol [12,51,52]. Pressurised liquid extraction method was previously elaborated for cocaine and benzoylecgonine extraction from coca leaves [53].

Until now, no comprehensive extraction optimisation of *Datura* alkaloids including traditional and modern methods (such as pressurised liquid extraction, PLE) was performed.

The crude extracts were typically purified by liquid–liquid partition and free bases were isolated with non-polar solvents (benzene, chloroform, etc.) [12]. Sometimes, Extrelut (Merck) cartridges were applied [13] or solid-phase extraction (SPE) was done usually on RP-18 columns [14–16,45]. A molecularly imprinted polymer (MIP) was also prepared for SPE of scopolamine from human urine and serum but low recoveries (46–79%) were measured [49]. Cation-exchange SPE was applied for the isolation of calystegines from plant samples, where they were retained by the charge of the secondary amino group [4,17].

GC methods are often applied for the analysis of tropane alkaloids [18–22]. Flame ionisation (FID) [18,22], mass spectrometry (MS) [19,20], phosphorus–nitrogen (NPD) [22] and electron-capture (ECD) [21] detection were used, and alkaloids could be derivatised with heptafluorobutyric anhydride [23]; *N*,*O*-bis(trimethylsilyl)-acetamide [24] or *N*-methyl-*N*-(trimethylsilyl)fluoro-acetamide [25]. Although using capillary columns in GC methods, efficient separation and high sensitivity can be obtained, tedious derivatization procedure makes some troubles. So HPLC methods are often considered in tropane alkaloids analysis.

Usually C18 stationary phase was applied for L-hyoscyamine and scopolamine separation [11,26–28,45] in gradient or sometimes isocratic mode. To improve the separation, sensitivity and peak symmetry ion-pair procedure was applied with sodium dodecyl sulphate [29], picric [30], *d*-10-camphorosulfonate [31] or methanesulfonic acid [32] additives to mobile phase as the ion-pair reagents. UV (or diode array detection, DAD) [11,26–28], RI (refractive index) [33], fluorescence [29] and MS [34] detectors for HPLC analysis tropane and related alkaloids were used. On-line LC–UV–MS and LC–NMR couplings enabled identification of tropane alkaloids in the extract of *Erythroxylum vacciniifolium* [50].

On-line quantification of tropane alkaloids by TLC methods on silica gel plates was also performed but derivatization step before densitometric evaluation was done with van Urk reagent [31], Dragendorff's reagent [11] or plates heated at 280 °C to produce fluorescence derivatives [35].

Over-pressure TLC (OPTLC) [39] and also high-speed counter-current chromatographic (HSCCC) technique [10] enabled preparative separation of the alkaloids.

These compounds were also analysed by capillary electrophoresis (CE) with UV [36] and MS [48] detectors as well as enantioselective capillary zone electrophoresis (CZE) [37,46,47] and micellar electrokinetic capillary chromatography (MECC) [38].

In our approach, comprehensive studies of L-hyoscyamine and scopolamine solid–liquid extraction (including PLE technique) from *D. stramonium* leaves were performed. The plant extracts were purified by newly elaborated procedure on mixed-mode reversed-phase cation-exchange SPE columns, and then qualitatively and quantitatively analysed by a new HPTLC-densitometry without derivatization step and RP-HPLC-DAD method omitting ion-par additives. Each method was validated and the quantitative results compared.

2. Experimental

2.1. Chemicals and plant material

The standards of scopolamine hydrochloride, L-hyoscyamine, scopolamine–*N*-oxide and scopolamine–*N*-methyl bromide were obtained from Sigma–Aldrich (St. Louis, MO, USA). Oasis MCX (60 mg, $d_p = 60\ \mu m$, 3 ml) mixed-mode cation-exchange solid-phase extraction columns were purchased from Waters (Milford, MA, USA). Tetrahydrofurane (HPLC grade) and HPTLC plates (20 cm × 10 cm, 0.25 mm thickness) coated with silica gel 60 F_{254} sorbent were from Merck (Darmstadt, Germany). Methanol, acetonitrile and 25% ammonia (each solvent was of HPLC gradient grade) were obtained from J.T. Baker (Gross-Gerau, Germany). Tartaric acid, 30% hydrochloric acid, 85% formic acid, acetone and chloroform (each solvent was of analytical grade) were from The Polish Reagents (POCh, Gliwice, Poland). Double-distilled water was used in all experiments. In the recovery studies, the mixture of four standards (scopolamine, scopolamine–*N*-oxide, L-hyoscyamine and scopolamine–*N*-methyl bromide) at the following concentrations, respectively, 5.55 mg/25 ml, 2.30 mg/25 ml, 5.00 mg/25 ml, 2.80 mg/25 ml was used. The investigated plant samples were correctly identified by the botanist (Mss Maria Być) and collected from Pharmacognostic Garden of the Medical University of Lublin in July 2000 (excluding second part of *Datura metel* and *Datura inoxia* seeds collected two years later). They included: *D. metel* seeds (harvested on the year 2000 and 2002), *D. metel* leaves (harvested on the year 2000), *D. stramonium* var. *stramonium* seeds and leaves, *D. stramonium* var. *godronii* seeds and leaves, *D. inoxia* seeds and herbs, *D. stramonium* var. *tatula* seeds and leaves, *Datura quercifolia* seeds, *Datura fastuosa* seeds and *D. stramonium* leaves. Voucher specimens of these plants are deposited at the herbarium of the garden.

2.2. Solid–liquid extraction procedures

2.2.1. Extraction on ultrasonic bath

One gram samples of dried and pulverised leaves of *D. stramonium* were placed in a 250-ml round-bottom flask together with 100 ml of 1% tartaric acid solution in methanol and extracted at room temperature on ultrasonic bath for 30 min (USTarR), at 40 °C (USTar40) or 60 °C (USTar60) for 15 min under reflux and also with pure methanol at 60 °C for 15 min