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Review

Tropane alkaloid analysis by chromatographic and electrophoretic techniques: An update☆

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ABSTRACT

Tropane alkaloids like atropine are antidotes applied against organophosphorus intoxications. Atropine is toxic itself and should be closely monitored during treatment. Hence, simple, fast, and sensitive determination methods for tropane alkaloids in serum are desirable. Mostly adopted methods of analysis are gas chromatography (GC); high performance liquid chromatography (HPLC), and capillary electrophoresis (CE). Various liquid and solid capillary fillings used in micellar electrokinetic chromatography, microemulsion electrokinetic chromatography, capillary electrochromatography, and enantioseparation provide high versatility to CE applications. In HPLC, specialised columns enhance separation efficacy. Ultraviolet light detection is common practise, but recently sensitivity and analyte identification were enhanced by coupling GC, HPLC, and CE to mass spectrometry. Apart from medical treatment, tropane alkaloids, cocaine in particular, are abused with various intentions. Forensic analysis of tropane alkaloids and their metabolites comprises the additional difficulty of unequivocal drug identification. Because of severe legal consequences, sophisticated analytical methods were developed and may provide additional techniques for therapeutic drug monitoring. Examples from forensic cocaine analysis and from doping analysis are included in this review.

Contents

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1. Introduction

1.1. Fields of tropane alkaloid analysis

For the treatment of intoxications with organophosphorus compounds, tropane alkaloids are important antidotes, given to victims of poisoning to counteract excessive acetylcholine effects. Dosage of therapeutic alkaloids in these cases is difficult, because the amount of organophosphorus toxicant that has been taken up and actually circulates in the body is usually unknown [1,2]. The tropane alkaloids are highly toxic themselves and should be dosed carefully; overdosing of the antidote may have a dramatic outcome. Also, individual patients appear to possess different sensitivity to tropane alkaloid treatment [3]. However, when no other means of monitoring are available, tropane alkaloid dosage is only adjusted following the visible intoxication symptoms. For practical atropine dosing schedules, a lack of evidence in the current recommendations leads to wide variations in clinical applications [4]. Therefore, simple, fast, and sensitive determination methods for alkaloids in serum are as highly desirable as for the organophosphorus toxicants.

Tropane alkaloids for therapeutic purpose are isolated from plants and applied as salts, e.g. atropine sulfate, or as semisynthetic derivatives such as homatropine bromide or *N*-butylscopolamine bromide (Fig. 1). The term tropane alkaloids derived from the 8-membered bicyclic ring system with a methylated bridge forming nitrogen that is called 8-methyl-8-azabicyclo[3.2.1]octane (IUPAC) or tropane (trivial name). Tropane alkaloids are obtained from Solanaceae, e.g., *Atropa belladonna*, *Hyoscyamus niger*, and *Datura stramonium*. The first pure compounds were atropine isolated from *Atropa belladonna* and hyoscyamine from *Hyoscyamus niger* [5]. Today, "atropine" is defined as the racemic mixture of (*S*)-hyoscyamine and (*R*)-hyoscyamine. (*S*)-hyoscyamine is genuine in plants and (*R*)-hyoscyamine forms under alkaline conditions. (*S*)-hyoscyamine possesses strong acetylcholine-inhibitory activity by blocking muscarine receptors, while the (*R*)-hyoscyamine is mostly inactive. Atropine, which is more often applied than (*S*)-hyoscyamine, exhibits approximately half of the pharmacological activity of (*S*)-hyoscyamine. Scopolamine, in contrast, is mostly applied as pure enantiomer, e.g. (*S*)-scopolamine bromide. Different tropane alkaloid derivatives and enantiomers also vary pharmacokinetically, so that actual blood levels will result from dosing and different turnover and excretion kinetics. High inter-individual variation in pharmacokinetic parameters was observed with tropane alkaloids after intravenous application [6]. When medication is to be monitored in short intervals, the major demands on the analytical methods are velocity and sensitivity. When drug metabolism in the body is to be investigated, degradation products with different physicochemical characteristics must be included into the analyses.

Tropane alkaloids, cocaine in particular (Fig. 2), apart from being applied for medicinal purposes are abused with various intentions. Forensic analysis of intoxication victims and of suspects comprises the additional difficulty of identification of the drugs and their metabolites, sometimes from a cocktail of various narcotics that were ingested [7]. In cases where a culprit is to be sentenced, analysis results must be quantitative and unequivocal in order to be litigable. Reliable drug identification and quantitation is also the task of doping analysis in sport contests [8]. Because of the high amount of money to be earned by drug dealers or by successful athletes, strategies to conceal drug trade and consumption are quite advanced. Because of the severe legal consequences and the high public concern, analytical methods to identify those drugs nevertheless are sophisticated and may provide techniques and strategies for therapeutic drug monitoring. Some examples from forensic analysis and from doping analysis are included in this review.

1.2. Intended coverage of the review

Irrespective of the purpose of the investigations, tropane alkaloids are predominantly analysed by gas chromatography (GC) and high performance liquid chromatography (HPLC). In addition, capillary electrophoresis (CE) is a versatile and fast developing analytical technique, and examples of CE separation of tropane alkaloids have been included in this review. Thin layer chromatography is of importance in fast analytical screening of complex samples, typically plant extracts, because they do not need intricate sample work up before separation. The technique is fast, robust, and costs for equipment and consumables are usually reasonable. Thin layer chromatography procedures for tropane alkaloids were included in a former review [9]. A recent monograph on thin layer chromatography in phytochemistry [10] and a review on advanced two-dimensional thin layer chromatography in the analysis of secondary plant metabolites include methods for tropane alkaloids [11]. In the present review, thin layer chromatography is not discussed in detail.

Methods for tropane alkaloid analysis in plant tissues and in human body fluids were recently summarised [12]. This review extensively describes procedures for tissue or fluid extraction and for sample preparation, which are of particular importance for all subsequent chromatographic measurements. Comprehensive comparisons of tissue work-up and sample preparation procedures for a large number of drugs and their metabolites allow generalisations that may be valuable for tropane alkaloid sample preparation in therapeutic drug monitoring by HPLC–MS [13] and in forensic urine samples by GC–MS [14]. Reduction of sample volume is considered as beneficial for both, faster analysis and the opportunity of direct coupling of the extraction device to the sample injector of the chromatography apparatus. Headspace air sampling for drugs like cocaine by direct introduction of polydimethyl siloxane fibres after solid phase microextraction of air was described [15] and belongs to the solvent-free techniques for extraction of analytes, which also have been summarised [16]. Microextraction by packed sorbent (MEPS) is the miniaturisation of conventional SPE packed bed devices from millilitre bed sizes to microlitre volumes. MEPS cartridges, like siloxane fibres, can be connected directly to a GC or a LC injector. The cartridges containing the solid packing material, after loading of the analytes and washing off contaminations, are placed as plugs between the glass syringe and the injection needle. The procedure can be fully automated [17]. A wide variety of sample preparation techniques before chromatographic analysis was comprehensively discussed in a recent survey [18]. As there are excellent summaries and surveys available in the recent literature, the present review does not focus on extraction and sample preparation.

Authors have tried to point out novel developments in tropane alkaloid chromatography and discussed velocity, specificity, and