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Screening Procedure for 30 Antihistamines H1 Using Capillary Gas Chromatography - Mass Spectrometry

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30 ANTİHİSTAMİNİN KAPİLER GAZ KROMATOGRAFİSİ -KÜTLE SPEKTROMETRİ YÖNTEMİYLE BELİRTİMİ

Özet

Bu çalışmada 30 adet antihistaminin pozitif-iyon elektron çarptırma ve pozitif-iyon kimyasal iyonizasyon kütle spektrumları sunulmuştur. İlaçlar alkali ortamda çekilenmiş, 25 m BP5 kapiler kolonda da ayrılmışlardır. GC alıkonma zamanları ve alıkonma indisleri SKF 525A iç standarda göre relatif olarak verilmiştir. Bulguları sunulan bu çalışmada antihistaminlerin çoğunun elektron çarptırma kütle spektrumları birbirine büyük ölçüde benzemekle birlikte, kimyasal iyonizasyon kütle spektrumlarının belirgin bir şekilde farklı olduğu saptanmıştır. Bu nedenle ilaç analizi yapan laboratuvarların her iki yöntemi birlikte kullanmaları önerilmektedir.

Summary

Positive-ion electron impact and positive-ion chemical ionization mass spectra of 30 antihistamines H1 are presented. Drugs were extracted under alkaline conditions, and separated on a 25 m BP5 capillary column. GC retention times are reported on both a retention indices basis as well as relative to SKF 525 A internal standard. While mass spectra were sometimes identical in electron impact mode, particularly for the ion m/z 58, using chemical ionization mode, characteristic ions were obtained.

Key words: *Antihistamines H1 - Forensic analysis - Capillary gas chromatography - Mass spectrometry*

INTRODUCTION

For over 40 years, the H1-receptor antagonists, first synthesized in 1942, have been widely used to relieve nasal itching, sneezing and rhinorrhea in patients with allergic rhinitis, and for relief of itching in patients with allergic skin disorders such as urticaria and dermatoses (1,2). Because of their anticholinergic properties, some of the antihistamines are used clinically for the treatment of motion sickness and vertigo. They are employed as both prescription and over-the-counter products. Accidental and intentional ingestion and overdose are common. Some fatalities were reported for diphenhydramine (3,4), cyclizine (5) or hydroxyzine (6,7).

Therefore, antihistamines are encountered frequently in clinical or forensic toxicological analysis. The detection of some drugs has been described, but none of these procedures allows the rapid and specific identification and differentiation of all antihistamines.

The present paper deals with positive-ion electron impact and positive-ion chemical ionization mass spectra of 30 antihistamines.

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EXPERIMENTAL

Chemicals and Reagents : Hydroxyzine hydrochloride (UCB Labs.), cyproheptadine base (Merck Sharp and Dohme Labs.), azatadine maleate (Schering-Plough Labs.), histapyrrodine base (Servier Labs.), clemastine fumarate (Sandoz Labs.), carbinoxamine maleate (Lafon Labs.), oxomemazine hydrochloride (Specia Labs.), chlorphenoxamine hydrochloride (Lucien Labs.), ketotifen fumarate (Sandoz Labs.), terfenadine base (Merrell Labs.) isothipendyl hydrochloride (Monot Labs.) and SKF 525 A hydrochloride, internal standard, I.S., (Smith Kline and French Labs.) were generously offered by the respective companies. Methaphenilene hydrochloride, methapyrilene base, cyclizine hydrochloride, chlorcyclizine hydrochloride, promethazine base, dimenhydrinate base, diphenhydramine base, doxylamine succinate, triprolidine hydrochloride, brompheniramine maleate, chlorpheniramine maleate, pheniramine maleate, pyrrobutamine phosphate, thenyldiamine hydrochloride, meclizine hydrochloride, pyrilamine hydrochloride, buclizine hydrochloride, phenindamine tartrate, and tripelennamine citrate were obtained using the Thetakit antihistamine standards TK-9000 FP (Theta Corporation). Hexane, isoamylalcohol, and methanol were HPLC grade (Merck). All other chemicals were analytical grade (Merck). Stock solutions of antihistamines (100 µg/ml, free base) and I.S. were prepared in methanol and stored at 4°C.

Chromatography : A model 8500 (Perkin Elmer) gas chromatograph with Ion Trap Detector (ITD), a capillary column, and a splitless inlet injection system was employed. The data system was an Epson PC AX computer. Data acquisition and manipulation were performed using standard software (Finnigan). The ITD was operated at 70 ev and 35 ev in electron impact mode and chemical ionization mode, respectively, with an ion source temperature of 200°C to 220°C, and m/z range from 40 to 500 amu. The electron multiplier voltage of the detector was set at 1450 and 1250 V, in electron impact mode and chemical ionization mode, respectively. A fused silica capillary column (SGE), BP-5 (cross-linked methylsilicone) 25 m x 0.22 mm, was used. The flow of carrier gas (helium, purity index N55) through the column was 2.2 ml/min and the head pressure on the column was maintained at 12 psi. The column oven temperature was programmed from an initial temperature of 60°C to 300°C at 30°C/min, and held at 300°C for the final 22 min. Splitless injection with a split value off-time of 1 min was employed. To produce chemical ionization, methane (purity index N55) was used.

Procedure:

a) Plasma, urine: Plasma or urine (1 ml) was pipetted into a 15-ml Pyrex centrifuge tube and followed by 0.5 ml saturated sodium carbonate buffer, 20 µl of I.S (10 µg/ml), and 5 ml of hexane-isoamylalcohol (99 : 1, v/v). After evaporating the organic mixture to dryness, the residue was dissolved in 25 µl of methanol, and 1 µl injected into the column.

b) Whole blood, tissue: Whole blood, or tissue homogenate (1 ml) was pipetted into a 15-ml Pyrex centrifuge tube and followed by 0.5 ml saturated sodium carbonate buffer, 20 µl of I.S. (100 µg/ml), and 10 ml of hexane-isoamyl-alcohol (99 : 1, v/v). After agitation and centrifugation, the organic phase was purified with an additional acidic extraction (1 ml of 0.1 N sulphuric acid). The aqueous acid layer was reextracted after addition of 0.5 ml of saturated sodium carbonate buffer, 0.5 ml of concentrated ammonia solution and 5 ml of hexane-isoamylalcohol (99 : 1, v/v). After agitation and centrifugation, the organic phase was evaporated to dryness; the residue was dissolved in 25 µl methanol, and 1 µl was injected into the GC column.

RESULTS and DISCUSSION

The GC properties of the examined antihistamines are summarized in Table I by retention indices and by relative retention times to SKF 525 A. All the tested antihistamines were subjectable to gas chromatography, without any detectable degradation by thermal decomposition. Retention indices provide preliminary indications for the possible presence of the compounds and may be useful to workers without a GC-MS facility.

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Table I. Antihistamines, molecular weight, retention indices on BP-5 column, and relative retention times to SKF 525 A.

Generic name	M.W.	Ret. Ind.	RRT-SKF
Azatadine	290	836	1.068
Brompheniramine	319	715	0.913
Buclizine	433	1806	2.307
Carbinoxamine	291	696	0.889
Chlorcyclizine	301	761	0.972
Chlorpheniramine	275	678	0.866
Chlorphenoxamine	304	704	0.899
Clemastine	344	833	1.064
Cyclizine	266	687	0.877
Cyproheptadine	287	822	1.050
Dimenhydrinate	470	631	0.806
Diphenhydramine	255	631	0.806
Doxilamine	270	648	0.828
Histapyrrodine	280	763	0.974
Hydroxyzine	375	1281	1.636
Isothipendyl	285	788	1.006
Ketotifene	309	986	1.259
Meclizine	390	1360	1.737
Methaphenilene	260	668	0.853
Methapyrilene	261	671	0.857
Oxomemazine	330	1079	1.378
Phenindamine	261	739	0.944
Pheniramine	240	618	0.789
Promethazine	284	787	1.005
Pyrilamine	285	754	0.963
Pyrrobutamine	312	841	1.074
Terfenadine	471	630	0.805
Thenyldiamine	261	678	0.866
Tripelennamine	255	669	0.854
Triprolidine	278	763	0.974

Mass/intensity data for 30 antihistamines are presented in Table II. In most compounds, the ions resulted from side chain cleavage in the ß-position to the side chain nitrogen gave base or intense peaks at m/z 58 or 72. These peaks were found in both electron impact and chemical ionization, but were generally lower in the latter mode.

Specific ions at m/z 96 and 99 were noted for compounds with methylpiperazine (cyclizine, chlorcyclizine) and methylpiperidine (azatadine, ketotifen, cyproheptadine) chains, respectively.

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The ion m/z 199, which is the phenothiazine ring, was present in the mass spectra of promethazine and isothipendyl, two phenothiazine antihistamines.

In the chemical ionization mode, [M + H] + quasi-molecular ions appeared for all compounds and constituted base peaks in several compounds. In this mode, the sensitivity was 10 to 30 fold lower than in the electron impact mode. For example, the detection limit of cyclizine in the electron impact and chemical ionization modes was 15 pg and 350 pg respectively.

Because of this prominent [M + H] + peak and the lack of extensive fragmentation, chemical mass spectra makes feasible the identification of targeted compounds even in mixtures that have not been well chromatographically resolved. Chemical ionization, used either by itself or to complement electron impact, is valuable in assuring accurate results.

As can be seen in Table II, the electron impact mass spectra of several antihistamines are virtually identical, which prevents identification especially when using the major ions m/z (58 or 72) in selected ion monitoring (SIM) Furthermore, the GC retention times of some compouds are virtually identical. Thus, a false positive response is quite possible. However, the chemical ionization mass spectra of these drugs are clearly differentiated.

Conclusion

The role of GC/MS in the drug testing field has grown significantely in the past few years. The combination of electron impact and chemical ionization to give complementary mass spectra, enables drug testing laboratories to generate accurate results.

Table II. Major ions in positive electronic impact and in positive chemical ionization mass spectra of 30 antihistamines H1.

Compound	EI mode	CI mode
Azatadine	232(100) 96(91) 246(83) 58(76)	291(100) 248(82) 290(69) 201(60)
Brompheniramine	58(100) 72(33) 167(16) 319(9)	321(100) 58(99) 276(67) 72(43)
Buclizine	231(100) 147(54) 285(44) 165(43)	201(100) 433(93) 321(81) 203(61)
Carbinoxamine	58(100) 71(43) 167(8) 81(5)	71(100) 72(100) 202(74) 58(73) 85(32)
Chlorcyclizine	56(100) 99(54) 70(47) 165(46) 58(26) 242(20)	302(100) 201(33) 99(25) 58(11)
Chlorpheniramine	58(100) 72(31) 275(24) 230(17)	58(100) 275(79) 276(35) 230(31) 72(30)
Chlorphenoxamine	58(100) 59(4) 77(3) 165(3)	58(100) 215(41) 72(30) 90(16) 59(13)

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Table II. (cont'd).

Compound	EI mode	CI mode
Clemastine	84(100) 128(24) 85(14) 82(10) 77(7)	215(100) 84(73) 128(72) 130(66) 345(48)
Cyclizine	56(100) 167(54) 99(51) 70(51) 165(36)	167(100) 266(69) 99(15) 170(15) 195(15)
Cyproheptadine	287(100) 96(91) 215(52) 57(51) 286(48)	288(100) 58(51) 289(43) 96(21)
Dimenhydrinate	58(100) 73(11) 165(5)	58(100) 167(65) 72(29) 256(20)
Diphenhydramine	58(100) 167(8) 59(6) 73(6)	58(100) 256(30) 59(15) 167(10)
Doxilamine	58(100) 71(54) 72(7) 182(6)	71(100) 182(66) 58(63) 271(50) 90(14)
Histapyrrodine	84(100) 91(67) 196(19) 65(14) 55(11) 280(7)	98(100) 84(50) 281(36) 99(33)
Hydroxyzine	210(100) 165(67) 45(56) 42(50) 56(46)	375(100) 201(73) 202(29)
Isothipendyl	72(100) 73(9) 86(6) 199(5)	86(100) 72(77) 241(53) 87(29)
Ketotifene	96(100) 309(95) 57(71) 73(61)	310(100) 96(89) 311(27) 97(22)
Meclizine	105(100) 189(66) 165(36) 79(16)	391(100) 201(85) 189(83) 389(68)
Methaphenilene	58(100) 97(43) 72(22) 202(20)	72(100) 58(75) 97(33) 202(21) 261(20)
Methapyrilene	58(100) 97(31) 72(22) 71(11) 217(5)	58(100) 72(93) 217(66) 262(46)
Oxomemazine	58(100) 73(5) 59(4) 281(2) 330(2)	58(100) 331(61) 332(24) 59(16) 100(16)
Phenindamine	260(100) 42(80) 261(57) 57(35) 203(17)	44(100) 262(86) 58(19)

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