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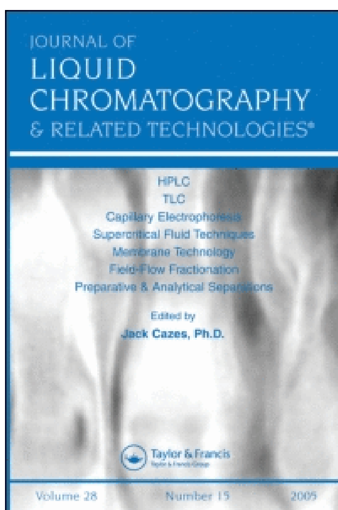

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Quantitative Liquid Chromatography, Thermospray/Tandem Mass Spectrometric (LC/TSP/MS/MS) Analysis of Some Tranquilizers of the Thioxanthene Group in Whole-Blood

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QUANTITATIVE LIQUID CHROMATOGRAPHY, THERMOSPRAY/TANDEM MASS SPECTROMETRIC (LC/TSP/MS/MS) ANALYSIS OF SOME TRANQUILIZERS OF THE THIOXANTHENE GROUP IN WHOLE-BLOOD

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ABSTRACT

A LC/TSP/MS/MS selected reaction monitoring (SRM) method in the daughter ion scan mode can be succesfully applied for the quantitative determination of members of the Thioxanthenegroup like Chlorprothixene,Flupenthixol,Thiothixene and Zuclopenthixol in whole-blood following a BondElut extraction.Detection limits as low as 100pg for Chlorprothixene varying to 2ng per injection for the other compounds were found (corresponding to 0.5 to 10ng per mL whole-blood).Sensitivity of the proposed method is of the same order of chromatographic methods with other detectors or a RIA method.

INTRODUCTION

Members of the Thioxanthenegroup like Chlorprothixene,Flupenthixol,Thiothixene and Zuclopenthixol are difficult to gaschromatograph.They all have in common

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large Retention Indices(1) causing on standard columns considerable analysis times.In order to overcome this problem we studied the use of liquid chromatography for the separation of the mentioned compounds,combined with thermospray tandem mass spectrometry for the selective and quantitative analysis of this type of drugs(2),like we did with a number of Benzodiazepines(3),several Explosives (4) and some representatives of the Methadone, Butyrophenone and Diphenylbutylpiperidine group(5).

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EXPERIMENTAL

Materials:

Pure substances of the following drugs Chlorprothixene, (CAS 113-59-7);Flupenthixol,(CAS 2709-56-0);Thiothixene,(CAS 5591-45-7)and Zuclopenthixol,(CAS 53772-83-1) were donated by the representatives of their producers Pfizer and Lundbeck.For structures see Figure 1.Blood used was outdated tranfusion blood and was frozen until used.Water was purified by the Milli Q/Organex System (Millipore).Acetonitrile,chloroform,dichloromethane and methanol were of HPLC and glass distilled grade (Rathburn).All other reagents were of analytical grade.Extractions were done by BondElut Certify columns(Varian)

Apparatus:

HPLC:A Waters 600-MS programmable pump,equipped with a U6K injector,was used to pump 0.6mL/min of a mixture

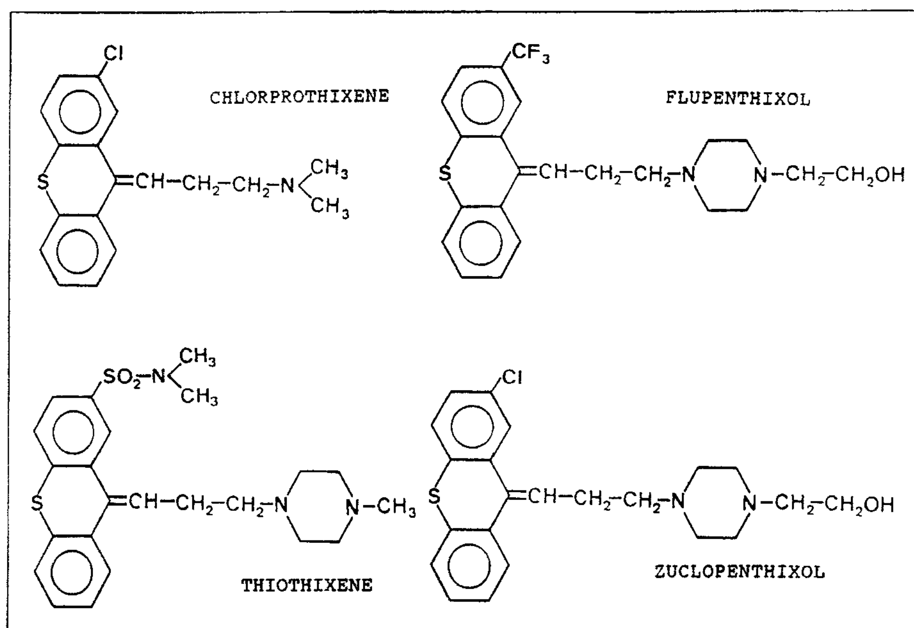

FIGURE 1
Structures of the compounds under investigation.

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(85:15) of acetonitrile and 50mM ammoniumacetate in water through a Hewlett-Packard HPLC cartridge column 5μm Asahipak ODP-50 4.0 x 125 mm .Post-column,an extra 0.6mL/min of 50mM NH4Ac in water was added by a Waters 590-MS isocratic pump for ionizing enhancement in thermospray applications.

MS:A Finnigan MAT TSQ 700 tandem quadrupole mass spectrometer,coupled to a DEC station 2100 was used.The liquid chromatograph was connected to the mass spectrometer by the Finnigan MAT TSP-2 interface.The operating

conditions of the interface,such as the repeller voltage,vaporizer temperature,source temperature and ionic strength of the eluent were all optimized for the different drugs and are given in Table 1.

MS/MS experiments in the daughter ion mode(6,7) were done with the triple-stage quadrupole mass spectrometer (Q1,Q2,Q3).In these MS/MS experiments,the $[M+H]^+$ quasi-molecular ion was chosen as precursor ion and selectively transmitted by Q1 for further collisional dissociation in Q2.Argon was used as the collisiongas with a collision chamber pressure of 2.5 till 4.0 mTorr (see Table 1).Varying collision offset voltages were applied to Q2.The collision activated dissociation(CAD) daughter ions thus obtained,were then analysed by scanning with the third quadrupole(Q3) over the mass range m/z 40-500(FullScan).In order to obtain optimum selectivity for the different drugs,not the FullScan but the Selected Reaction Monitoring(SRM) technique(2,6) was applied.In this case,only one special ion was allowed to pass the third quadrupole(Q3).Then selectivity is extraordinarily increased(8),as was prospected for the sensitivity,by extremely suppressing the noiselevel. Collision offset voltage,argon pressure and MSMSC factor(a correction factor for increasing the transmission of ions in the MS/MS mode) were all optimized,and the most intense fragment ion in the MS/MS spectrum was chosen for SRM experiments(see Table 1).

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TABLE 1

HPLC/MS/MS Parameters and Detection Limits SRM method (S/N ≈ 3) for Reference Solutions of some members of the Thioxanthene group.

TSP conditions:Repeller,70 V;Vaporizer temperature, 130-135°C;Source temperature,200°C;Filament off.
MS conditions:Multiplier Voltage,1500V;Dynode power, 15 kV;Scantime,1.20 sec;MSMSC factor 0.

Substance SRM m/z's	Collision Offset(V)	pArgon (mTorr)	R.T (min)	Dect.Lim. On Column
Chlorprothixene 316 - 271	-17.5	3.5	4.50	0.1ng
Flupenthixol 435 - 265	-35.0	4.0	3.15	2.0ng
Thiothixene 444 - 335	-15.0	2.5	2.50	5.0ng
Zuclopenthixol 401 - 128	-17.5	3.0	3.40	2.0ng

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Reference solutions:

Stock solutions of the different substances were prepared once a week by dissolving 10mg of the pure sub-substances in 10mL of methanol. From these,diluted solutions of 1,10,100 and 1000ng per mL of the different compounds were prepared by addition of methanol;10µL were injected in the chromatograph.All reference solutions were stored in glass vials with teflon coated silicone rubber-lined crimp caps.Whole-blood was spiked by adding a quantity of the drug in methanol to blank

blood,taking care that the amount of methanol did not exceed 2%.The blood was spiked with Chlorprothixene in the concen-tration range of 1 to 200ng per mL whole-blood with seven different concentrations.

Sample treatment:

The extraction procedure is the one we use in this laboratory in routine determinations of a general unknown in blood by HPLC with UV detection.Extractions were done with BondElut Certify 3cc columns(Varian). Preconditioning of the column was done with 2mL of methanol followed by 2mL of a 0.1 M phosphate buffer of pH 6.0.Care was given to wetting of the column until the prepared blood sample was brougth on column.The preparation of the blood sample was done by diluting in a polypropylene tube of 1mL of blood with 6mL of 0.1M phosphate buffer of pH 6.0.After vortexing and sonification the solution was centrifugated and the clear solution transferred to the column.Then the column was rinsed with water,followed by 1mM acetic acid(pH 3.3), afterwards the column was dried by suction.Elution was done first by 2mL acetone:chloroform 50:50,giving an acidic fraction,followed by elution with 3mL of freshly prepared ethylacetate:ammonia solution 98:2, giving the neutral and basic fractions used in the experiments Of the last fraction the solvent was evaporated at 40°C under a gentle stream of nitrogen.The extract is dis-

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solved in 50µL methanol and an aliquot of this solution (10µL) is injected into the chromatographic system.

RESULTS AND DISCUSSION:

In Table 1 the thermospray and MS/MS parameters are given.In optimizing the thermospray parameters,it was found that moderately low repeller voltages could be used in all experiments and that a rather high vaporizer temperature was very benificial in terms of signal to noise ratio.Application of lower temperatures of the vaporizer gave less noise, but also a far lesser signal. Variations of the collision gas pressure were of relatively little importance regarding sensitivity of the SRM method,but the values given for the voltages of the collision offset and the MSMSC factor are of utmost importance.Slight variations of these parameters give undesirably large changes in sensitivity.

In Table 2 a comparison is given for the detection limits in the FullScan and the SRM(daughterion) mode.In contrast to the findings with the Benzodiazepines(3),the explosives(4) and some members of the Methadone,Butyrophenone and the Diphenylbutylpiperidine group(6) the sensitiviy of the SRM method was in the same order as was the FullScan method.So no enhancement of sensitivity by using the SRM method was found, only selectivity was improved.We found that the detec-

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TABLE 2

On column Detection Limits in ng for FullScan and SRM (S/N ≈ 3) for Reference Solutions for some members of of the Thioxanthenegroup.

Substance	FullScan	SRM
Chlorprothixene	0.25	0.1
Flupenthixol	1.0	2.0
Thiothixene	1.0	5.0
Zuclopenthoxol	1.0	1.0

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tionlimit in the SRM mode was moderately influenced by other ions in the original Q1 spectrum being present together with the nearly always "dominating" protonated molecular ion(3,4,5,8).Furthermore it appeared,that although the parameters for the collision activation process are chosen with utmost care;the ideal collision activation process for analytical quantitative purposes, giving only one dissociation product from the starting product $[M+H]^+$ could not be adjusted.Even when varying the parameters for the collision activated dissociation process very carefully,the protonated molecular ion gave always rise to quite a lot of dissociation products. In our view the piperazine group plays in these processes an important role.Because of these two interacting processes the dectionlimits in the SRM mode cannot surpass the detection limits of the FullScan mode. The detection limits found for these members of

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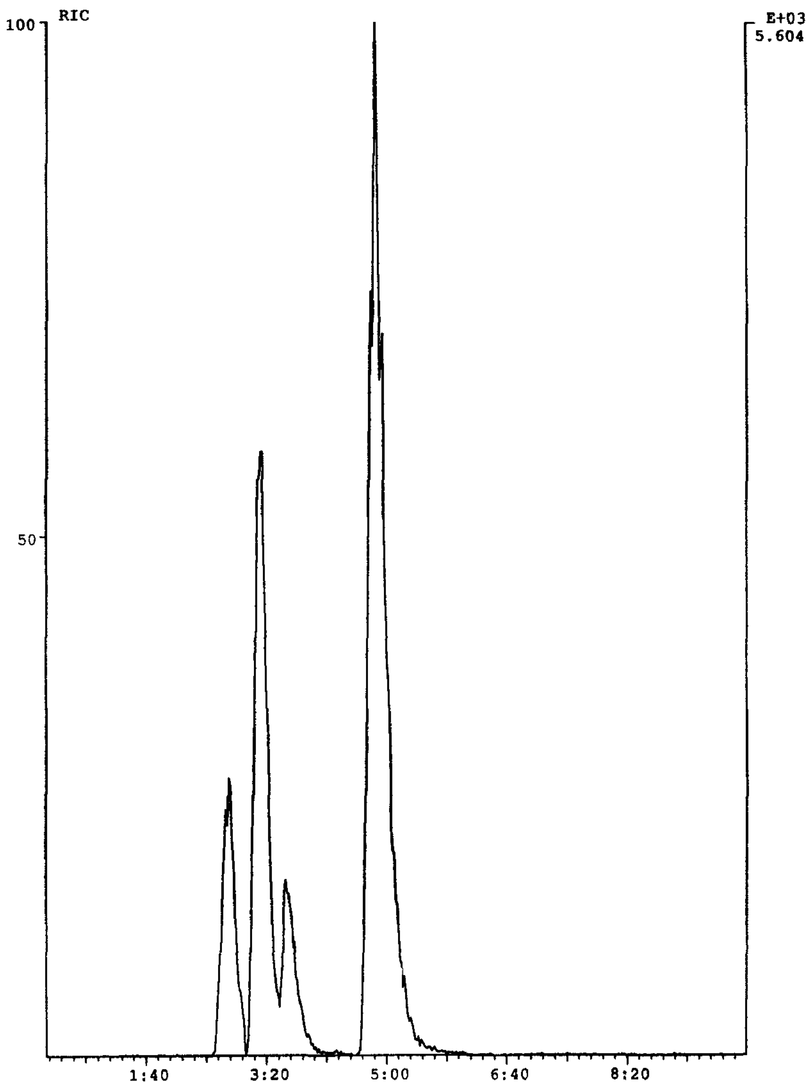

FIGURE 2 .
Chromatogram of the four thioxanthenes.For chromatographic conditions see Text.For mass spectrometric conditions see Text and Appendix 1.Sequence of elution:Thiothixene,Flupenthixol,Zuclopenthixol and Chlorprothixene On column about 10ng of each compound.

the Thioxanthene group were of the same order as the values usually found in litterature(1) using other analysis methods.The extraction method,as described in sample treatment, was checked for Chlorprothixene as model compound,as it can be thought that the other Thioxanthenes have more or less the same extraction properties.Spiked whole-blood samples,in the range of 1 to 200ng per mL blood were extracted by the BondElut method. For reference solutions and spiked blood extracts calibration curves could be constructed,with slopes of 0.25 and 0.24 counts.µl.10^4.ng^{-1} respectively and with intercepts of -2.12 and 1.01 counts.10^4 in this concentration range. From the slopes a recovery of 95 % could be calculated.The regression coëfficients of 0.982 and 0.996 were found.A standard deviation of 7.5% was found for spiked blood samples at a concentration of ≈10ng/mL (n=6).In Table 1 the values for the $[M+H]^+$ and the preferential ions in the MS/MS spectra are given. All the selected m/z values from the Table are tested for interference with the possible presence of ions from extracted blood.No such interferences were found.In Figure 2 a chromatogram of a separation of the four thioxanthenes is given using the procedure described in Appendix 1.About 10ng of each compound was injected.

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APPENDIX 1.

```
NAME: psycho

PAGE 1
prof;coll=70;vaphtr=135
delayscan %1,%2,%3
dau 444,334,336,1.2,-15
while rt<3.00
 go;stop;end
dau 435,264,266,1.2,-35
while rt>=3.00 & rt<3.50
 go;stop;end
dau 401,127,129,1.2,-18
while rt>=3.50 & rt<4.50
 go;stop;end
dau 316,270,272,1.2,-17.5
while rt>=4.50 & rt!=0
 go;stop;end
off
cent

------- PAGE -------      -------- LINE --------      PF4 :EDIT/CMD
PF6:SAVE    PF8:RUN       SPF6:SAVE    SPF8:RUN       PF10:ABORT
PF7:REST    PF9:KILL      SPF7:REST    SPF9:KILL      PF11:CANCEL

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REFERENCES:

1.A.C.Moffat,J.V.Jackson,M.S.Moss,B.Widdop and E.Greenfield,eds.,Clarke's isolation and identification of drugs,The Pharmaceutical press,London,1986,pp462,483, 628 and 1021.

2.W.M.A.Niessen and J.v.d.Greef,LiquidChromatography-Mass Spectrometry,Marcel Dekker,New York,1992,pp.157-203.

3.A.M.A.Verweij,P.J.L.Lipman and P.G.M.Zweipfenning, Forens.Sci.Int.54:67-74(1992).

4. A.M.A.Verweij,P.C.A.M.de Bruyn,C.Choufoer and P.J.L. Lipman,Forens.Sci.Int.60:7-13(1993).

5. A.M.A.Verweij,M.L.Hordijk,P.J.L.Lipman,J.Anal.Tox. in press.

6. J.Yinon,MS/MS techniques in Forensic Science.In A. Mealy and R.L.Williams(eds.),Forensic Science Progress Vol.5,Springer Verlag,Berlin,1991,pp.1-29.

7. C.E.M.Heeremans,Thesis,University of Leiden,1990.

8. A.M.A.Verweij and P.J.L.Lipman,J.Chrom.653:359-362 (1993).

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