QUALITATIVE AND SEMIQUANTITATIVE TLC ANALYSIS OF CERTAIN PHENOTHIAZINES IN HUMAN PLASMA

LUMINIȚA BLENDEA*, DAN BĂLĂLĂU, CLAUDIA MARIA GUȚU, MIHAILA ILIE, DANIELA LUIZA BACONI

"Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy, Toxicology Department, 6 Traian Vuia, 020956, Bucharest, Romania
*corresponding author: ldblendea@yahoo.com

Abstract
Phenothiazines have been reported to be rather often the cause of acute intoxications, either in overdoses or in associations with other medicines and/or alcohol. The paper presents a simple thin layer chromatographic method to qualitatively and semiquantitatively assay chlorpromazine, thioridazine and levomepromazine in normal human plasma spiked with the three substances, following liquid – liquid extraction with dichloromethane. Several mobile phases were tested and methanol : n-butanol (60:40), with 0.1 M NaBr was considered the best to be used in the determination. The analytes were found in the samples with a recovery coefficient of 42%, 78% and 67% for chlorpromazine, thioridazine and levomepromazine, respectively.

Keywords: chlorpromazine, thioridazine, levomepromazine, TLC analysis, toxicity

Introduction
Acute intoxications are often due to deliberate or accidental improper use of medicines prescribed by the physician; among the most incriminated such agents are benzodiazepines, barbiturics and phenothiazines [1,2,3]. Establishing the xenobiotic that generated the intoxication is a rather difficult task, as clinical signs are often similar, and the chemical agent is established generally from the declarations of the intoxicated patient or the person(s) that found him (her). One of the best methods to attest the intoxication agent is the gas-chromatography – mass spectrometry (GC-MS) method, which produces a “fingerprint” of the substance that can be further identified using mass
spectra libraries [4,5]. Unfortunately, the cost of the equipment is rather high and also highly specialised and skilled personnel is needed.

Thin layer chromatography (TLC) provides a simple and not expensive alternative to the qualitative analysis in the toxicology laboratory; it can also be successfully used for a robust evaluation of the analyte amount in the sample, in different biological matrices [6,7].

The paper presents a TLC method to assay certain phenothiazines (chlorpromazine, thioridazine, levomepromazine) in human plasma.

Materials and methods

Standard solutions

The following stock solutions were prepared: chlorpromazine hydrochloride (C) conform to Ph.Eur., bought from Office Chimique Certa S.P.R. L., Belgium, 1 mg/mL in methanol HPLC grade (Sigma), thioridazine hydrochloride (T), from Sigma, 1 mg/mL in methanol HPLC grade (Sigma), levomepromazine hydrochloride (L), injection solution in NaCl 0.1%, 1 mg/mL in methanol HPLC grade (Sigma).

Reagents and biological samples

The following reagents were basically used for the determinations: dichloromethane (Sigma), chloroform (Chimopar S.A.), 6 M sodium hydroxide (Merck) solution in distilled water, 8% sodium bicarbonate (Merck) in distilled water, sodium chloride, normal human plasma from the “C.T.Nicolau” National Haematologic Institute.

TLC

Precoated silicagel glass, aluminium and plastic plates, 60 F254, 20 x 20 cm, from Merck, were used. For the mobile phases, the following solvents and reagents have been used: methanol (HPLC, Sigma), ethanol 96% (Chimopar S.A.), ethyl acetate (p.a., Chimopar S.A.), ammonia 25% (Chimactiv S.A.), chloroform (Chimopar S.A.), n-buthanol (Merck), NaBr (Chimopar S.A.), propionic acid (Merck). For the preliminary tests literature references have been used [4,8].

Equipment

The experiments were performed using the following devices: Linomat 5 (Camag, Switzerland), computer assisted, semi-automatic sample spotting device, vertical developing glass tank with lid, TLC scanner 3 (Camag, Switzerland), computer assisted, winCATS planar chromatography manager software (Camag, Switzerland), videodocumentation station Reprostar 3 (Camag, Switzerland), vortex mixer Genie 2 (Cole Parmer), Sigma 2-16K refrigerated centrifuge (Germany), sample concentrator (nitrogen flow), Techne Dry-Block DB-3D (Bibby Scientific Inc., England).
Procedure

We spotted on the same plate the standard solutions (aliquots of 2 to 20 μL) in parallel with the samples prepared as further described. The plates were developed with the mobile phase in a developing vertical tank, previously saturated with the mobile phase vapors, in a dark place. When the mobile phase front reached about 1 cm from the plate edge, the plates were extracted from the tank and let still at dark for the evaporation of the mobile phase. The examination of the plates was further performed using UV light at λ=254 nm, in reflectance mode.

The samples were prepared as following: to 1 mL of human plasma, 100 μL 6 M NaOH, 0.8 g NaCl, 2 mL dichloromethane and appropriate aliquots of standard solutions or methanol (for blank) were added. The mixture was mildly vortex mixed for 15 minutes, then centrifuged at 3000 rpm for 10 minutes, 24º C, the supernatant was discarded, the organic phase was collected and evaporated to dryness under nitrogen flow. The residue was dissolved in 2 mL of methanol and further used for the assay.

Analysis

The separation results were expressed as Rf values, automatically computed by the winCATS software according to the equation (1):

\[ R_f = \frac{l_{\text{compound}}}{l_{\text{solvent}}} \]  

(1)

where \( l_{\text{compound}} \) represents the migration distance of the compound from the origin, and \( l_{\text{solvent}} \) represents the migration distance of the solvent front from the origin.

The semiquantitative analysis was performed by means of peak area for each spot, provided by the winCATS software of the densitometer. The densitometry was performed in absorption (reflection) at 254 nm.

Results and discussion

The tests performed on plastic and aluminium precoated plates were irrelevant, so only the glass silicagel precoated plates have been further considered for analysis.

Generally, the tested systems could not properly separate all three compounds, best results being obtained for the systems presented in Table I. From the data presented in the table, a better separation of the compounds seems to be achieved with system 1 (ethyl acetate : methanol : ammonia, 85:15:5). However, as for that system chlorpromazine and thioridazine could not be separated (figure 1) and the dilution of the samples resulted
only in a weaker and a more diffuse spot for both chlorpromazine and thioridazine (figure 1), we selected system 3 (methanol : n-buthanol, 60:40, with 0.1 M NaBr) to be used for further investigations. Figure 2 presents an example of a 3D chromatogram of C, T and L solutions as such and extracted from the human plasma have been spotted on the plate.

<table>
<thead>
<tr>
<th>System</th>
<th>Mobile phase</th>
<th>R_f values</th>
<th>C</th>
<th>T</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ethyl acetate : methanol : ammonia (85:15:5)</td>
<td>0.174±0.008</td>
<td>0.204±0.014</td>
<td>0.289±0.002</td>
<td></td>
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<tr>
<td>2.</td>
<td>Chloroform : ethanol (90:10)</td>
<td>0.213±0.016</td>
<td>0.21±0.017</td>
<td>0.297±0.037</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Methanol : n-buthanol (60:40), with 0.1 M NaBr</td>
<td>0.402±0.011</td>
<td>0.50±0.011</td>
<td>0.47±0.01</td>
<td></td>
</tr>
</tbody>
</table>

1 All mixtures of solvents are expressed as parts, v/v.

Table 1
R_f values obtained for the best systems used

Figure 1
Chromatogram of C, T, L and mixture (M) obtained with system 1 (table I); different amounts of all substances have been spotted.
As can be seen, the three substances are rather well separated, but the spots are diffuse. The obtained Rf for the three extracted substances were 0.41, 0.49 and 0.47 (for chlorpromazine, thioridazine and levomepromazine, respectively), thus permitting the accurate identification of the three substances.

For the semiquantitative assay of the extracted C, T and L from spiked human serum samples, a calibration curve was drawn as peak intensity (expressed in absorption units) vs. the amount spotted for the standards (figure 3), then the amount of the extracted C, T and L was computed based on the parameters of the regression line for each substance (table II).
Table II

<table>
<thead>
<tr>
<th>Substance</th>
<th>Computed amount (μg)</th>
<th>Recovery coefficient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ce</td>
<td>0.025</td>
<td>42%</td>
</tr>
<tr>
<td>Te</td>
<td>0.047</td>
<td>78%</td>
</tr>
<tr>
<td>Le</td>
<td>0.040</td>
<td>67%</td>
</tr>
</tbody>
</table>

As can be seen, an acceptable recovery coefficient has been obtained for thioridazine, but the extraction method seems to be less efficient for chlorpromazine and levomepromazine.

Conclusions

Chlorpromazine, thioridazine and levomepromazine were assayed from mixtures and from human plasma spiked samples by thin layer chromatography.

The best system used was TLC pre-coated silicagel F$_{254}$ glass plates and the mixture methanol : n-buthanol (60:40), with 0.1 M NaBr as mobile phase. Chlorpromazine could be well separated from the other two substances in these conditions, but thioridazine and levomepromazine even if rather well resolved, can overlap in amounts exceeding 10 μg spotted. The analytes could be identified separately from spiked human plasma samples following extraction with dichloromethane.
The analytes were found in the samples with a recovery coefficient of 42% for chlorpromazine, 78% for thioridazine and 67% for levomepromazine.

References

8. *** Camag Application Notes No. A-45.1, HPTLC determination of free chlorpromazine in urine and detection of its metabolites

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