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Peak Purity Assessments in Chromatography: A Case Study of Chlorpromazine Hydrochloride

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This work examines the applicability of different peak purity and/or homogeneity deconvolution algorithms for the evaluation of chromatographic purity and/or homogeneity in chlorpromazine hydrochloride, in the order of the algorithm's increasing complexity. The methods are examined for usefulness with a view to possible routine application in a busy quality assurance laboratory include chromatographic peak assessment, spectral normalization, absorbance ratios and chromatographic derivatives.

Key Words: Chlorpromazine hydrochloride, chlorpromazine impurities and chromatographic peak purity.

INTRODUCTION

The phenothiazine dopamine antagonist, chlorpromazine hydrochloride is widely used as an antipsychotic agent. Its oxidation products, chlorpromazine sulphoxide and chlorpromazine sulphone (Figure 1), induce photosensitivity reactions when consumed. For this reason, most pharmacopoeia define limits for the related impurities permitted in the pharmaceutical dosage forms. Although high performance liquid chromatography (HPLC), can be used to determine the drug content in the dosage form, the phenomenon of chromatographic peak cross-over and peak-peak overlap or migration of related impurities have been reported [1].

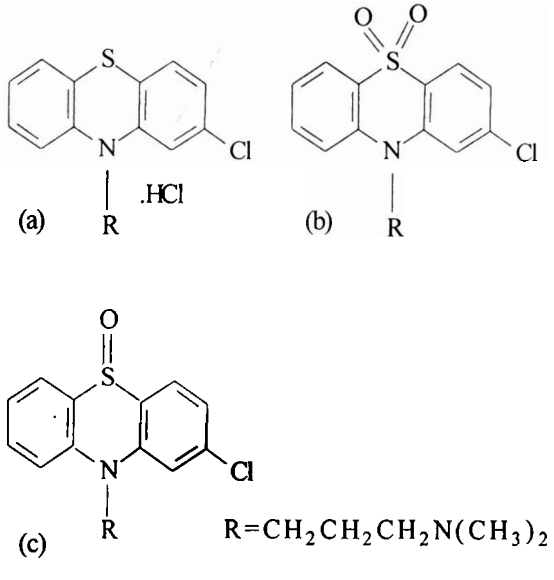

Figure 1. The structures of (a) chlorpromazine hydrochloride, (b) chlorpromazine sulfoxide and (c) chlorpromazine sulfone.

The occurrence of this phenomenon, which is dependent on the pH and composition of the mobile phase, makes it imperative for the pharmaceutical analyst to be able to routinely carry out a rapid assessment of the chromatographic peak purity of the component of interest. The co-eluting components in the system provide a suitable challenge with which to test the different peak homogeneity algorithms.

The utilization of multichannel detectors such as the linear photodiode array detectors [2-4] provide good opportunity for the application of several digital algorithms for the examination of chromatographic peak purity. This is attributed to the capacity of the linear photodiode arrays to collect spectro-chromatographic data in the absorbance (A), wavelength (λ) and time (t) matrix. Such instruments have in-built routine software that is easy to apply during post collection data manipulations. Examples of such in-built software packages include: 3D pseudo-isometric projections [5-10], two dimensional contour diagrams [4,7,11,12], spectral normalization, absorbance ratios of chromatograms at a pair of wavelengths [13-18], spectral and chromatographic derivatives [19-22] and the absorbance difference method of spectral suppression [5,6,8,23-25].

This study examines the possibility of using a number of deconvolution algorithms for the purpose of rapid routine assessment of chromatographic peak purity of the component of interest. The algorithms examined can be utilized for post collection analysis of data using any computerized analytical system equipped with appropriate software.

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EXPERIMENTAL

Instrumentation

The Multichannel Diode-Array Detector (DAD), HP 1040A with an 8-channel data processing unit was interfaced with an HP-85 microcomputer to facilitate the rapid acquisition of the spectro-chromatographic data. In addition, the detector was equipped with a 4.5 µl volume flow cell. This system was connected to an HP 9121 dual disc drive, an HP 7470A plotter and an ink-jet printer (Hewlett-Packard, Avondale, USA). An LDC-constametric 3000 dual piston pump (LDC-Milton Roy, Rivera Beach, F1 USA) was employed. The HPLC column used for the experiment was a 100 x 4.6 mm stainless steel packed with 5 µm CPS-Hypersil particles.

Reagents and Chemicals

The acetonitrile and methanol of HPLC grade were obtained from Rathburn (Walkerburn, UK). The ammonium carbonate, was of analytical grade and obtained from Sigma Chemical Ltd. Co. (St. Louis, MO, USA). Chlorpromazine hydrochloride (CPZ-HCl) and chlorpromazine sulphoxide (CPZ-S) were from May and Baker (Rhône Poulenc, Dagenham, UK). Concentrated hydrochloric acid and ammonium hydroxide were analytical grade reagents.

Preparation of the Mobile phase

The mobile phase consisted of acetonitrile-methanol-10 % ammonium carbonate (40:30:10, v/v). The pH of the mobile phase was adjusted with 0.1 M ammonium hydroxide or 0.1 M hydrochloric acid in order to achieve different degrees of resolution between the CPZ-HCl and CPZ-S. The mobile phase flow rate was 1 ml/min.

Preparation of samples

Solutions of CPZ-HCl (32.76 µg/ml) were injected into the column in duplicate and spectrochromato-graphic data was collected at four different wavelengths, namely λ=220.4, 240.4, 250.4 and 272.4 nm. The resulting peak heights or peak areas were obtained by the resident integration software programme. The experiment was repeated after addition of CPZ-S (25.64 µg/ml) solution to each solution of chlorpromazine hydrochloride.

RESULTS AND DISCUSSION

Chromatographic peak assessment

The manipulation of pH of the mobile phase facilitated the co-elution of the CPZ-HCl and CPZ-S to a near symmetrical single peak (Figure 2). Different degrees of chromatographic peak overlap provided appropriate challenges to the peak purity/homogeneity assessment algorithms being examined for suitability in rapid routine application in a busy quality assurance laboratory. Chromatographic peak assessment was carried out by extracting the normalized spectra at different retention times (upslope, apex and downslope) of the elution peak for the candidate substance. When the extracted spectra are overlaid, they do not superimpose, but instead one obtains individual spectra corresponding to both CPZ-HCl and CPZ-S, thus indicating coelution of the two compounds. The results indicate that it is possible to detect the presence of CPZ-S in the CPZ-HCl. When spectral profiles of co-eluting components differ significantly from each other, this method is adequate for the detection of contaminants.

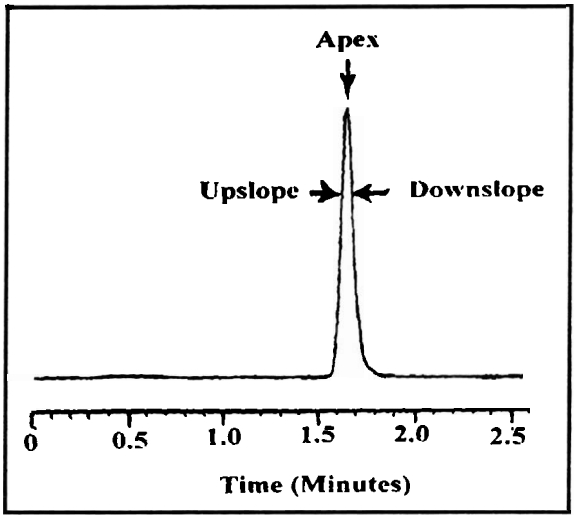

Figure 2: Liquid chromatogram of a mixture of chlorpromazine hydrochloride and chlorpromazine sulphoxide

The use of absorbance ratio values for the identification of an analyte and detection of possible contaminants, has been extensively discussed elsewhere [26]. A ratiogram presents the ratio of elution profiles for the substance when collected at two different wavelengths. Figure 3 shows the liquid chromatogram and ratiogram of CPZ-S.

The flat slope indicates that the ratio of

absorbance at 272 nm and 250 nm (A $\lambda_{272}/\lambda_{250}$) is constant and hence there are no detectable impurities in the sample.

By contrast, the ratiogram in figure 4 deviates from the horizontal and indicates that the peak is not homogeneous. Indeed, the sample in this case is a mixture of CPZ-HCl and CPZ-S.

Therefore, it is possible by this method to detect the presence of an impurity when the analyte and impurity spectra differ significantly.

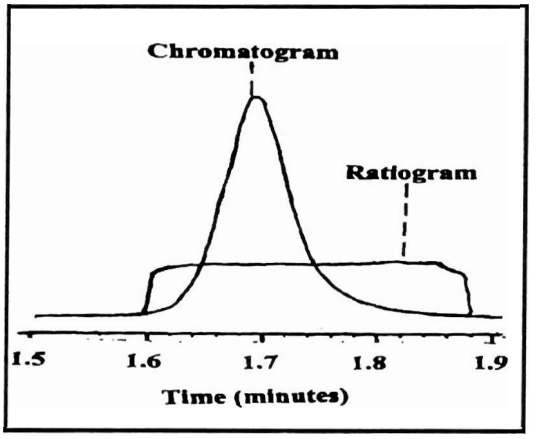

Figure 3: Liquid Chromatogram and ratiogram of chlorpromazine sulfoxide.

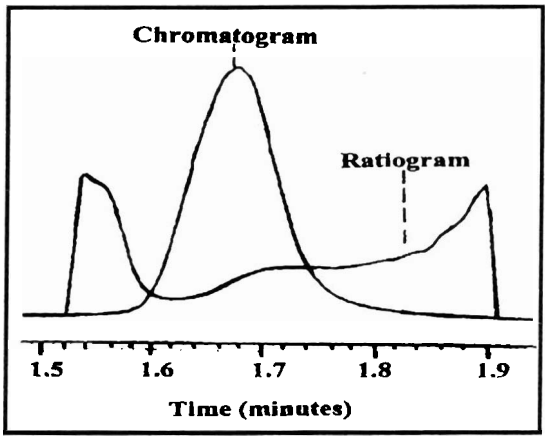

Figure 4: Liquid chromatogram and ratiogram of a mixture of chlorpromazine sulphoxide and chlorpromazine hydrochloride

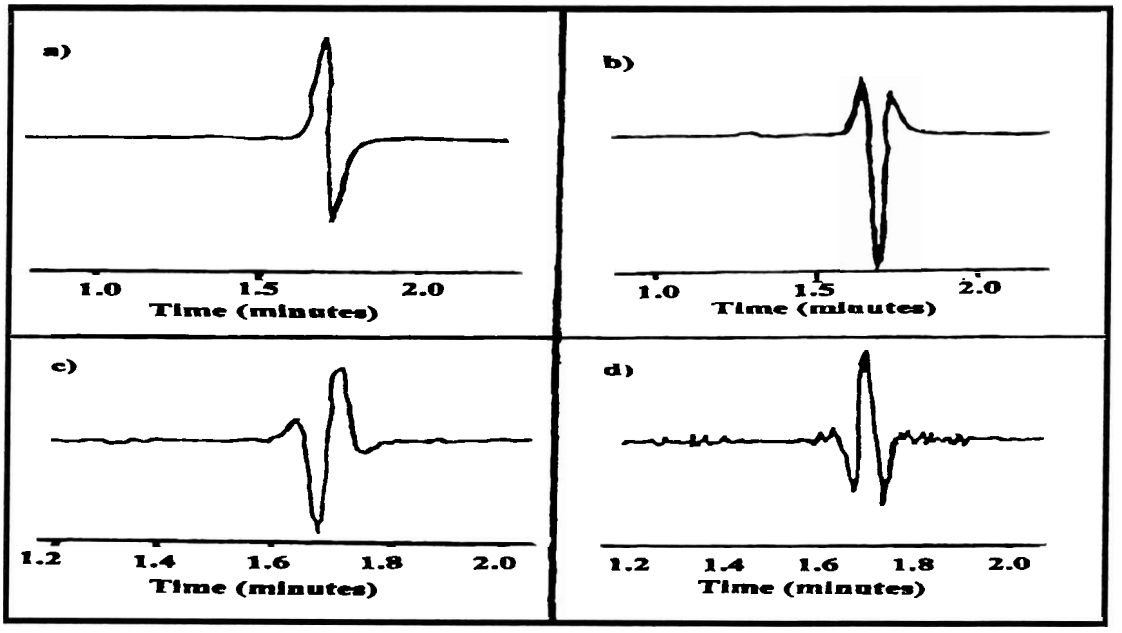

Figure 5: First (a), second (b), third (c) and fourth (d) derivatives of a spectrochromatographic signal obtained from chlorpromazine sulphoxide

Chromatographic Derivatives

Chromatographic differentiation (1st to 4th order) of the signal was carried out using resident programmes on the HP1040M detector for the spectrochromatographic signals obtained from the pure sample of the CPZ-S (25.64 μg/ml). These are compared with those obtained from a similar manipulation of the signal obtained from a mixture containing both CPZ-HCl and CPZ-S. The results show that the zero order (ordinary) and first order chromatograms (Figure 5a) do not detect any difference in the signals due to CPZ-S alone and due to a mixture of CPZ-HCl and CPZ-S (Figure 6a). The second order chromatogram derived from a CPZ-S/CPZ-HCl mixture gives a slight kink, which is an indication of inhomogeneity (Figure 6b). However, with the third order chromatographic signal derivatives the contamination is clearly detectable as a kink and there is a significant difference between the signals due to the pure CPZ-S (Figure 5c) and that obtained from the mixture (Figure 6c). It was observed that with the fourth chromatographic derivative, there are two distinct peaks for the signal due to the mixture (Figure 6d) while the CPZ-S displayed only one peak (Figure 5d). Therefore, the 4th order chromatographic signal could be used successfully to detect the presence of chlorpromazine sulphoxide in therapeutically useful hydrochloride salt in a pharmaceutical dosage form.

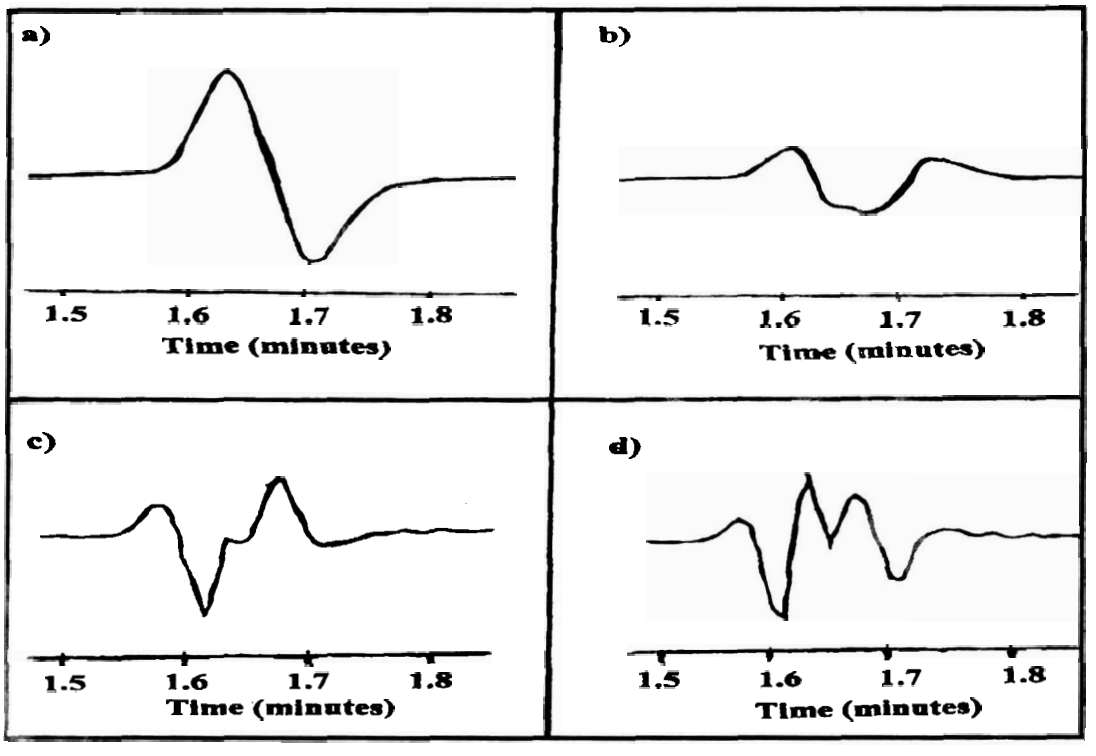

Figure 6: First (a), second (b), third (c) and fourth (d) derivative of a spectrochromatographic signal obtained from a mixture of chlorpromazine sulphoxide and chlorpromazine hydrochloride

CONCLUSION

These simple algorithms can readily be applied in computerized systems for post collection analysis of the data obtained. Their simplicity and ease of application using resident software will assist the quality assurance personnel in busy analytical laboratories to determine with certainty the peak purity/homogeneity of components after separation.

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