A NEW CONCEPT FOR TESTING CHROMATOGRAPHIC PEAK PURITY; APPLICATION TO DETERMINATION OF THE MAIN INGREDIENTS IN TEA EXTRACTS

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Abstract

A new concept was applied to test the spectral purity of chromatographic peaks. The proposed concept is based on the application of the orthogonal functions (P_1 and P_2) to the absorption spectra recorded at five different time intervals throughout the eluted chromatographic peak. Ratios of strong peaks of the p_1 and p_2-convoluted absorption spectra were computed for the investigated samples and for standard solutions of the corresponding reference materials. Such ratios are considered spectrophotometric characteristics for each compound, independent of concentration. The percentage deviation in these ratios between the samples and the reference materials exceeded 2% in case of impure peaks. For further assessing the peak purity, the obtained convoluted curves were examined. The pure peaks share the same characteristic spectral features and intersect at the same wavelengths. This new concept was applied to determine the peak purity of the main alkaloids in black, green and white tea extracts which contain different interfering structurally related substances. The results have been compared to those obtained using the Agilent Chemstation software. Same results were obtained using both methods. The proposed concept could be considered a universal technique for peak purity assessment in any quality control laboratory.

Introduction

The spectrochromatograms extracted at different time intervals across an HPLC peak are UV absorption spectra of the eluted compounds. The shape of such spectra is dependent on the structure of the eluted compound while the intensity of the absorption is dependent on the concentration of such compound at any time throughout the elution of the HPLC peak. The co-elution of an absorbing compound and/or impurity along with the main compound would contribute to the absorption of the main compound. Ratios of strong peaks of p_1 and p_2 convoluted absorption spectra are considered spectrophotometric characteristics of each compound, independent of concentration and these ratios could be applied to assess chromatographic peak purity.

Tea (*Camellia sinensis* L) is one of the most widely consumed nonalcoholic beverages due to its medicinal, refreshing and mild stimulant effects. Four types of tea are produced from the leaves of *Camellia sinensis* based on treatment of the harvested leaves. There are white tea (harvested leaves buds with white trichomes, non-fermented or semi-fermented), green tea (non-fermented), Oolong tea (semi-fermented) and black tea (fermented). The differences among brands of tea arise from processing, growth conditions, and geography. Tea leaves are an important source of methylxanthines alkaloids caffeine (CF), theobromine (TB) and theophylline (TP). Methylxanthines are used as therapeutic agents and have been utilized in many pharmaceutical formulations as analgesics, diuretics and bronchodilators. CF is the major component of methylxanthine alkaloids that are formed in tea leaves while TB and TP are minor components.

A wide variety of analytical methods have been reported for the analysis of CF, TP and TB in food, drinks and tea samples. Most of these studies are based on HPLC.

The aim of the present work is to introduce a new concept for testing the spectral purity of the chromatographic peaks through the application of the orthogonal functions (P_1 and P_2). The orthogonal functions and the ratios of the convoluted absorption spectra were computed at the peaks optima to identify the investigated compounds and ensure the absence of interference from other biosynthesized structurally related compounds.

Experimental

Instrument

Agilent high performance liquid chromatographic system (1200 series) comprised of a quaternary pump with rheodyne 7725i 7-port sample injector and photodiode array detector. The device is operated by Agilent chemstation software for LC.

Chemicals & Reagents

Pharmaceutical grades of CF, TP and TB were kindly supplied by Amriya for pharmaceutical industries, Alexandria, Egypt. Acetonitril (HPLC grade) was purchased from Merck. Sodium acetate, glacial acetic acid, anhydrous sodium sulphate, lead acetate and sodium carbonate were all of analytical grade. Tea samples were purchased from a local market.

Preparation of tea extract

Samples of 1 g of black, green and white tea were accurately weighed and boiled for 30 min in 100 mL of distilled water. Water evaporation was compensated during boiling. The obtained infusions were then filtered and the filtrate was quantitatively transferred into a 100-mL volumetric flask and diluted to volume with water. A 0.5 mL aliquot of lead acetate (10 % w/v) was added to a 25 mL aliquot of the sample to precipitate tannins and the mixture was stirred for 3 min at room temperature. The solutions were filtered and 0.1 mL of sodium carbonate (10 % w/v) was added to the filtrate to remove excess lead acetate. The mixture was stirred for 2 minutes at room temperature then filtered and volumes were adjusted to 100 mL with distilled water.

Chromatographic conditions

Aliquots of standard CF, TP and TB solutions containing 15 µg mL^{-1} were injected into the HPLC column concurrently with the tea extracts using the chromatographic conditions described in the BP under the monograph of TB or TP "test for related substances". The chromatograms were extracted at 272 nm and integrated using Agilent Chemstation software.

Results & Discussion

The peaks of CF, TP and TB of the standard mixture shared the following observations which are considered as characteristic features for a pure peak:

(a) The UV-absorption spectra extracted at different time intervals throughout each eluted peak are similar to that extracted at the peaks apex (spectrum apex).

(b) The wavelengths of absorption and convoluted curve optima of the UV-absorption spectra extracted at different time intervals of the elution of each peak are almost identical.

(c) The convoluted orthogonal absorption curves (p_1 and p_2) of the UV-absorption spectra obtained at different time intervals of the elution of each peak intersect at the same wavelengths.

The purity of the HPLC peaks of CF, TP and TB reference standard solutions and standard mixture were tested using Agilent chemstation software. The purity factor is within the calculated threshold limit indicating that the eluted peaks are pure. The chromatograms of black, green and white tea extracts showed peaks corresponding to CF and TB obtained using the specified chromatographic conditions (see experimental).

Figure (1) shows the HPLC chromatogram of green tea extract (a representative example of the 3 studied types of tea). The spectrochromatograms of these peaks (Fig. 1) revealed that the peak of CF is pure due to the following observations:

a) The spectrochromatograms extracted at different time intervals throughout the eluted peak are similar to that extracted at the peak apex (spectrum apex) (Fig. 1).
b) The convoluted orthogonal absorption curves (p_1 and p_2) of the spectrochromatograms obtained at 5 different time intervals of the elution of the peak intersect at the same wavelengths (Fig. 1)
c) The ratios of the absorbance and orthogonal (p_1 and p_2) optima of the UV-absorption spectra extracted at different time intervals of the eluted peak are similar to those of standard CF with RSD % values less than 2% (Table 1).

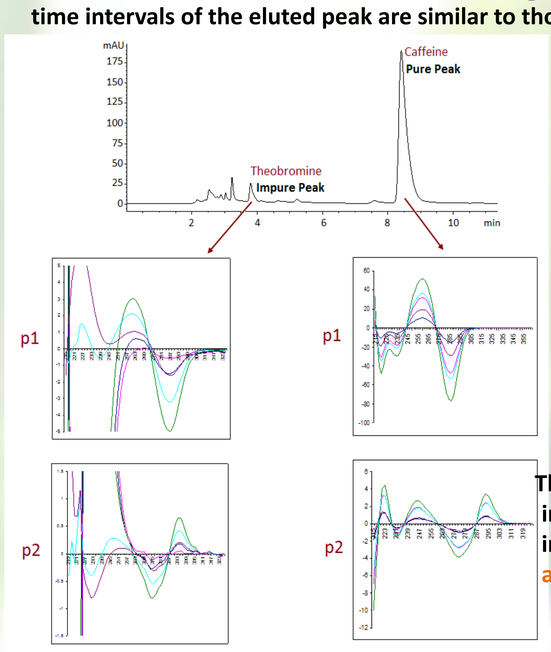

Fig. 1. Convoluted orthogonal absorption curves obtained at five time intervals across the eluted chromatographic peaks of theobromine and caffeine in green tea extract

Table 1. Ratios of the orthogonal optima (p_1 and p_2) of the spectrochromatograms extracted at 5 different time intervals for the eluted CF peak obtained from HPLC of the extract of green tea sample

	$A_{273/245}$		$p1_{287/259}$		$p2_{275/293}$	
	Reference 15 µg mL^{-1}	sample	Reference 15 µg mL^{-1}	sample	Reference 15 µg mL^{-1}	sample
Mean	3.392	3.328	1.502	1.502	1.117	1.126
SD	0.028	0.047	0.002	0.001	0.009	0.003
RSD %	0.825	1.412	0.133	0.067	0.806	0.266
% deviation	-	1.887	-	0	-	0.799

The proposed method for testing peak purity was applied. The results indicated that the eluted peak of TB in the extracts of tea samples is impure due the the following observations:

a) The spectrochromatograms extracted at 5 different time intervals throughout the eluted peak are different and have not the same characteristic spectral features (Fig. 1)
b) The convoluted orthogonal curves (p_1 and p_2) of the spectrochromatograms obtained at 5 different time intervals of the elution of the peak do not intersect at the same wavelengths (Fig. 1).
c) The ratios of the absorbance and orthogonal optima (p_1 and p_2) of the spectrochromatograms extracted at different time intervals of the eluted peak are not similar to those of standard TB with RSD % values deviated by more than 2 % (Table 2).

The purity of the HPLC peaks of CF and TB in tea samples were tested using Agilent chemstation software. The results indicated that the peak due to CF is pure and that due to TB is impure (Fig. 2). The purity factor of CF peak is within the calculated threshold limit indicating that the eluted CF peak is pure. The purity factor of TB peak exceeds the calculated threshold limit indicating that the eluted TB peak is impure.

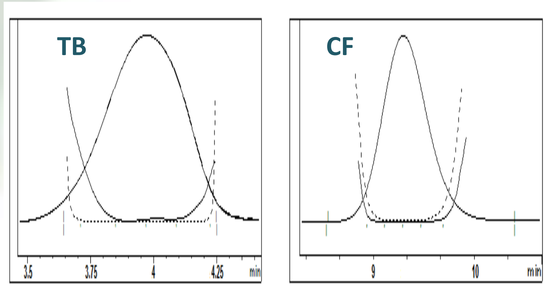

Fig. 2. Purity plots of TB and CF peaks obtained from HPLC of green tea sample showing the autothreshold line (—) and the purity line (···).

Table 2. Ratios of the orthogonal optima (p_1 and p_2) of the spectrochromatograms extracted at 5 different time intervals for the eluted TB peak obtained from HPLC of the extract of green tea sample

	$A_{273/245}$		$^1p_{287/259}$		$^2p_{275/293}$	
	Reference 15 µg mL^{-1}	sample	Reference 15 µg mL^{-1}	sample	Reference 15 µg mL^{-1}	sample
Mean	3.392	3.328	1.502	1.502	1.117	1.126
SD	0.028	0.047	0.002	0.001	0.009	0.003
RSD %	0.825	1.412	0.133	0.067	0.806	0.266
% deviation	-	1.887	-	0	-	0.799

Conclusion

The application of orthogonal polynomials method to the spectrochromatograms extracted throughout the elution of an HPLC peak could be used successfully for testing the spectral purity of the resolved HPLC peaks.

References

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