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COMPUTER SIMULATED MODEL OF SECOND ORDER DERIVATIVE OF ABSORBANCE SPECTRUM OF A MIXTURE FOR DETERMINATION OF CONCENTRATION OF ITS INDIVIDUAL COMPONENTS.

Pharmacology

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ABSTRACT

The use of spectrophotometer in analytical chemistry of mixtures is limited by overlap of absorbance spectrum of the components of the mixture. The absorbance of a mixture at a particular wavelength is the sum-total of the absorbance of all the analytes in the mixture at the same wavelength. Determination of concentration of one analyte in the mixture will be overestimated due to absorbance of other analytes (herein known as interferents). The estimation method can be imprecise (due to varying content of interferents) and always inaccurate (due to presence of interferents). In such spectrophotometric method involving multiple analytes, derivative spectroscopy can be used to ameliorate failed accuracy validation. It uses first or higher derivative of absorbance with respect to wavelength for both qualitative and quantitative discrimination. The derivative bands are sharper and separate the overlapping bands. The peaks of derivative spectra can be used to construct the calibration curves for determination of the concentration of the analyte of interest in the mixture. In this article, we describe a computer simulated model of derivative spectroscopy for estimation of concentration of the individual drugs in a solution.

KEYWORDS

Derivative spectroscopy, Spectrophotometry, analytical chemistry.

INTRODUCTION

According to the WHO Guide to Good Prescribing, monitoring of treatment is an integral part of any rational pharmacotherapy exercise. Generally, we use well defined clinical and laboratory parameters for monitoring efficacy and safety of drugs. In absence of such correlates, the monitoring of serum drug levels is a very useful tool in determining the course of treatment [1]. However, therapeutic drug monitoring (TDM) relying on HPLC/LCMS is a resource intensive facility unavailable in many settings. Spectrophotometry, despite being hierarchically lower in the field of analytical chemistry, is a robust and cost-effective alternative. It can deliver limited but quality TDM services in a basic hospital laboratory [2]. In simple words, the principle of spectrophotometry (based on Beer Lamberts law) is that the intensity of light absorbed through a solution is quantitatively related to the concentration of the analyte in the solution. Within the range of the linear part of the relation, a calibration curve (CC) is plotted with incremental standard concentrations on the x-axis and the corresponding absorbance on the y- axis. The wavelength at which maximum absorbance is observed for a particular combination of solute and solvent is known as lambda max (λ max). The unknown concentration of a substance is determined with the help of its standard CC. Validated spectrophotometric methods for drug estimation in biological samples are available in erstwhile as well as contemporary literature [3]. The first and vital step in these methods is the preferential extraction of the analyte into a suitable solvent. In absence of interfering substances in the sample, the analyte may be detected and quantified directly with the help of the recorded absorbance (at λ max) and the CC. Alternatively, the errors due to interfering molecules can be eliminated by selectively converting the analyte into a new reaction product. The concentration of the unknown is determined with the CC of the reaction product. Nevertheless, overlapping of spectra due to interferents is a significant impediment to the specificity of any spectrophotometric method. Chromatography methods are free from such interference because the sample is run in a medium where the components of a mixture get separated and detected in tandem. In spectrophotometric method involving multiple analytes, derivative spectroscopy can be used to ameliorate failed accuracy validation. It uses first or higher derivative of absorbance with respect to wavelength for both qualitative and quantitative discrimination. The derivative bands are sharper and separate the overlapping bands. The peaks of derivative spectra can be used to construct the CC. In severely overlapping spectra, complex statistical models based on Partial Least Square regression, Principal Component Analysis or Net Analyte Signal are developed and validated for determining the analyte Concentration [4]. Although the current gold standard for TDM are chromatographic methods, spectrophotometry with proper techniques and validation can be a viable alternative in a resource limited setting.

MATERIALS AND METHODS

Range of wavelength for spectral scanning- 300 to 700 nm

Solution – Mixture of Drug A and Drug B in a suitable solvent (Solvent X)

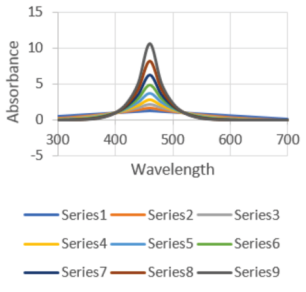
Lambda max for Drug A in solvent X= 460 nm

Lambda max for Drug B in solvent X= 480 nm

Computer generated data:

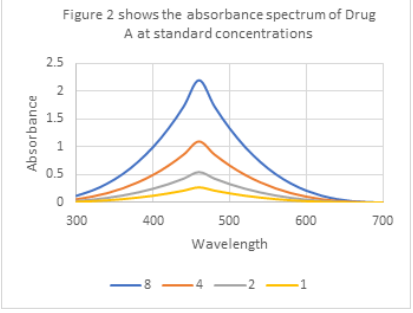
The absorbance spectrum of a chemical compound in a particular solvent is its characteristic property. The absorbance varies as an exponential function of the wavelength. The absorbance spectrum of hypothetical molecules with incremental exponents but having the same lambda max at 460 nm is shown in figure 1

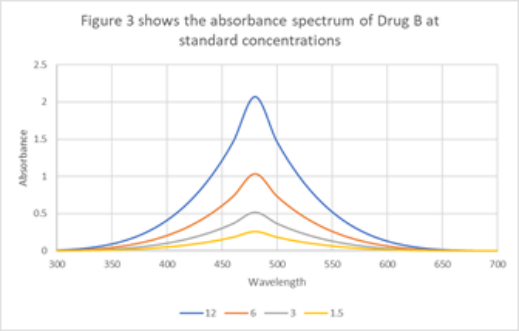
Figure 1: Absorbance spectrum of chemical compounds are its characteristic property even with same lambda max. The rate of change of absorbance with wavelength determines this unique property



A common baseline computer simulated data raised to the exponent of 3 and 4 was used to generate the absorbance spectrum at standard concentrations for Drug A and Drug B respectively (Table 1, Figure 2 and Table 2 and Figure 3 for drugs A and B respectively). Calibration curves were constructed using the simulated data (Table 3, Figure 4 and Figure 5 for drugs A and B respectively).

Figure 2 shows the absorbance spectrum of Drug A at standard concentrations





Wavelength in nm	Absorbance at Standard Concentrations of Drug A			
	8	4	2	1
700	0.001	0.0005	0.00025	0.000125
680	0.008	0.004	0.002	0.001
660	0.027	0.0135	0.00675	0.003375
640	0.064	0.032	0.016	0.008
620	0.125	0.0625	0.03125	0.015625
600	0.216	0.108	0.054	0.027
580	0.343	0.1715	0.08575	0.042875
560	0.512	0.256	0.128	0.064
540	0.729	0.3645	0.18225	0.091125
520	1	0.5	0.25	0.125
500	1.331	0.6655	0.33275	0.166375
480	1.728	0.864	0.432	0.216
460	2.197	1.0985	0.54925	0.274625
440	1.728	0.864	0.432	0.216
420	1.331	0.6655	0.33275	0.166375
400	1	0.5	0.25	0.125
380	0.729	0.3645	0.18225	0.091125
360	0.512	0.256	0.128	0.064
340	0.343	0.1715	0.08575	0.042875
320	0.216	0.108	0.054	0.027
300	0.125	0.0625	0.03125	0.015625

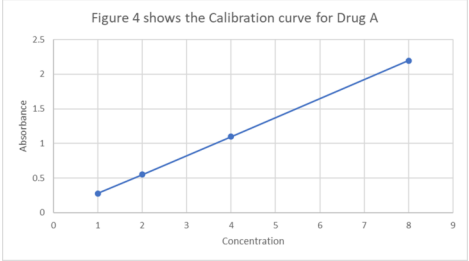
Table 1 shows the computer generated absorbance values of Drug A at standard concentrations

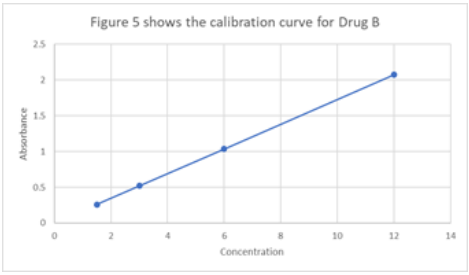
Wavelength in mm	Absorbance at Standard Concentrations of Drug B			
	12	6	3	1.5
700	0.0001	0.00005	0.000025	0.0000125
680	0.0016	0.0008	0.0004	0.0002
660	0.0081	0.00405	0.002025	0.0010125
640	0.0256	0.0128	0.0064	0.0032
620	0.0625	0.03125	0.015625	0.0078125
600	0.1296	0.0648	0.0324	0.0162
580	0.2401	0.12005	0.060025	0.0300125
560	0.4096	0.2048	0.1024	0.0512
540	0.6561	0.32805	0.164025	0.0820125
520	1	0.5	0.25	0.125
500	1.4641	0.73205	0.366025	0.1830125
480	2.0736	1.0368	0.5184	0.2592
460	1.4641	0.73205	0.366025	0.1830125
440	1	0.5	0.25	0.125
420	0.6561	0.32805	0.164025	0.0820125
400	0.4096	0.2048	0.1024	0.0512
380	0.2401	0.12005	0.060025	0.0300125
360	0.1296	0.0648	0.0324	0.0162
340	0.0625	0.03125	0.015625	0.0078125
320	0.0256	0.0128	0.0064	0.0032
300	0.0081	0.00405	0.002025	0.0010125

Table 2 shows the computer generated absorbance values of Drug B at standard concentrations

Drug A		Drug B	
Concentration	Absorbance	Concentration	Absorbance
8	2.197	12	2.0736
4	1.0985	6	1.0368
2	0.54925	3	0.5184
1	0.274625	1.5	0.2592

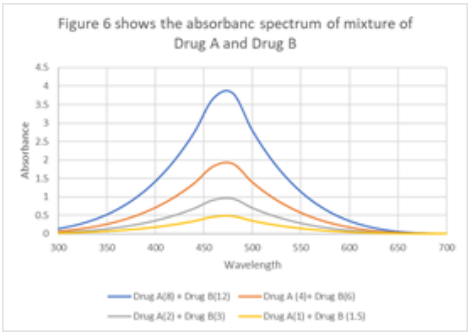
Table 3 shows the absorbance values of drugs A and B at standard concentrations at lambda max





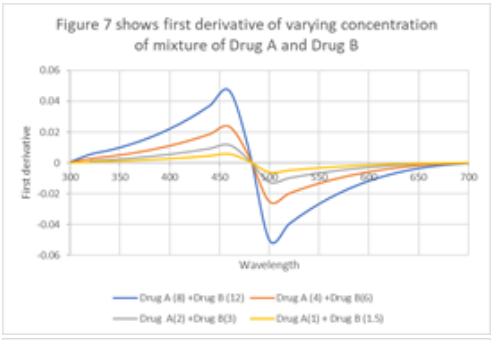
Absorbance spectrum of mixture of Drug A and Drug B:

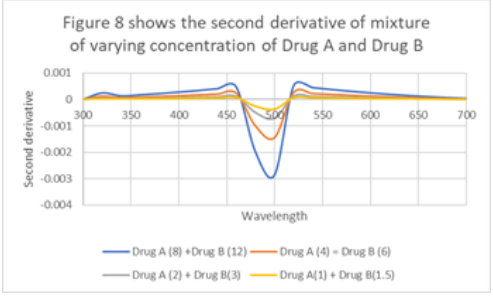
The absorbance spectrum of mixture of Drug A and Drug B at varying concentration was constructed by summing up the individual absorbance of the two drugs at specific wavelengths (Figure 6)



Derivative Spectroscopy

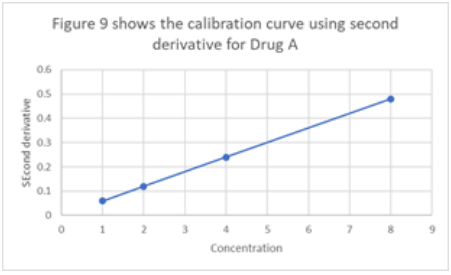
First and second order derivatives were calculated for the absorbance of the mixture of the drugs (Figure 7, Figure 8)

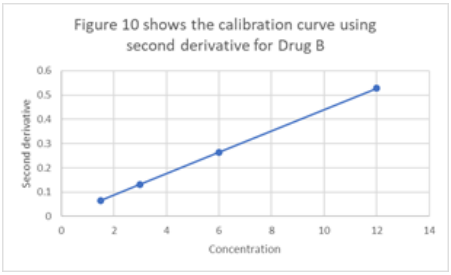




Calibration curve of second derivative

The second derivative has two peaks which corresponds with the individual peaks of Drug A and Drug B in isolated solutions. Calibration curves were constructed for the two drugs with the values at the peaks of the second derivative at standard concentrations of the two drugs (Figure 9, Figure 10)





Determination of concentration of unknown in mixture

The concentration of unknown is determined using the value of second derivative of the unknown and deriving it from the regression equation of the standard curve.

DISCUSSION

Derivative spectroscopy is an important technique of extracting information from spectra composed of overlapping bands. It is obtained by first or a higher order mathematical derivative of absorbance against wavelength ($dA/d\lambda$). Derivative spectro photometry has been applied to many chemical systems, such as foods, pharmaceuticals, cosmetics, and environmental samples [5]. Derivative transformation allows elimination of broad band interferents and discriminates subtle spectra features, thus increasing the sensitivity and specificity in mixtures analysis. In our computer simulated model, we used computer generated data to derive the first and second derivative that can be used for determination of unknown concentration of the analyte. Simulated derivative ratio spectra are run in two steps. First, the derivative model is simulated using computer generated data. The method is then validated by preliminary model data and by application to the real world data. Recently, Zhang et al. introduced derivative spectrophotometry for the quantification of overlapping peaks in capillary electrophoresis (CE) [6]. However, the derivative mode has lesser reproducibility compared to direct spectrophotometric detection. Sensitivity of the two modes are comparable but separation efficiency of derivative spectroscopy is much higher as the peak is much sharpened [4]. Derivative spectrophotometry saves cost and time since only few optimizations of separation conditions for normal calibration curve are required to demarcate the overlapping spectrums. It plays an important role in analysis of pharmaceuticals and has emerged as an integral part of the drug development process. It has a huge impact on the technological and scientific progress in this field. It is therefore imperative to develop analytical methods to determine the concentration of drugs in quality control as well as manufacturing phase pharmaceutics. The determination of concentrations of analytes in biological samples is also achievable by complex spectrophotometric methods. For example, UV spectrophotometric and derivative spectrophotometric methods have been widely used for fluoroquinolones and are covered under a recent review published by Kaur et al [7]. Similarly, 1st and 2nd derivatives of the ratio spectra and measurement at zero-crossing wavelengths in a mixture of aspirin, Salicylic acid, paracetamol was used for simultaneous determination of the three drugs in their synthetic mixtures and dosage forms. The ratio spectra were obtained by dividing the absorption spectrum of the mixture by that of one of the components in the mixture [8]

CONCLUSION

Computer simulated models can be used to save time and money in analytical methods. Using the data available from pre-existing literature and database, the analytical behaviour of mixtures can be predicted and derivatives can be obtained. Methods which fail in simulated model may not be tried in real world experiments. Successful models can be used for simultaneous determination of two or more compounds in the same sample without attempted chemical separation. In this way, under computer-controlled instrumentation, derivative techniques play an important role in the analysis of mixtures by UV–vis molecular absorption spectrophotometry. The approach is very useful in the resolution of band overlapping in quantitative analysis. Derivative techniques have proved to be very useful in the resolution of binary and ternary mixtures.

Limitations:

The derivatisation of zero-order spectrum produces ($n + 1$) new peaks for n-derivative order. Therefore, the resulted derivative spectrum is more complex than the parent-one. If the original spectrum shows m maxima, the number of new extremes in the generated derivative spectrum is increased by a factor of m. The obtained derivative spectrum becomes complex. There is increase in signal to noise ratio resulting in significant errors. This corollary shows that the numbers of derivatives in spectrophotometry used for resolving of ternary, quaternary or more complex mixtures are limited.

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