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Short communication

Chromatographic and UV-VIS and IR spectral methods for qualitative and quantitative analysis of some synthetic bromo-flavone derivatives

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1. Introduction

Cascaval and co-workers [1,2] had synthesised a series of halogenated flavone derivatives. Microbiological and capillary protector tests and their very acceptable 50% lethal dose (LD_{50}) revealed that these compounds seem to be interesting and are suitable to be conditioned in pharmaceutical forms.

Flavone derivatives are implicated in anti-HIV 1 action [3] and are adenosine A_1, A_{2a} and A_3 receptor ligands. However, further studies concerning flavonoide derivatives must be carried out in order to establish detailed structure-activity relationships.

Our study aims the physical and chemical characterisation and quantitative assay validation, in order to establish the dosage form, the stability and the pharmacokinetics of two bromo-flavone derivatives: 3-bromo-6-methyl-2′-hydroxy-flavone (**I**) and 5,7-dibromo-6-methyl-2′-hydroxy-flavone (**II**) (Fig. 1).

TLC, UV-VIS and IR spectral characterisation were used to investigate methods for qualitative and quantitative determination of these compounds from pharmaceutical forms.

Kieselgel GF_{254} thin layer chromatographic studies, using acetonitrile-water-formic acid (25:4:2, v/v/v) or benzene-acetonitrile (3:1, v/v) as solvent systems, reveal the possibility of identification and purity control of these compounds. These two derivatives have characteristic UV-VIS peaks: (**I**) at 251, 271, 322 and 402 nm and (**II**) at 255, 277, 324 and 406 nm and IR peaks: (**I**) at

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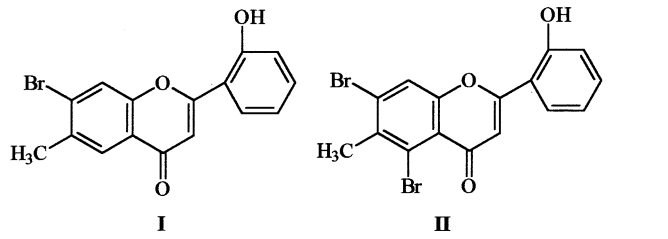

3-bromo-6-methyl-2'-hydroxy-flavone 5,7-dibromo-6-methyl-2'-hydroxy-flavone

Fig. 1. The structure of bromo-flavone derivatives (**I**) and (**II**).

1680, 1584, 1408, 1348, 1312, 1264, 1168, 1136, 816 and 752 cm^{-1} and (**II**) at 1668, 1632, 1584, 1456, 1408, 1312, 1264, 1216, 1168, 764, 624 and 592 cm^{-1}. Two UV and VIS spectrometric methods for quantitative determination were proposed, using peaks from 322 and 402 nm for compound (**I**) and peaks from 255 and 406 nm for compound (**II**).

2. Materials and methods

2.1. TLC studies:

Thin layer chromatographic tests were performed on HPTLC Fertigplatten Kieselgel 60 F_{254} (Merck, Darmstadt, Germany), using as solvent the following systems: acetonitrile-water-formic acid (25:4:2, v/v/v) (solvent A) or benzene-acetonitrile (3:1, v/v) (solvent B). In both cases, the samples were prepared in methanol, 0.04 mg ml^{-1}. Visualisation of chromatograms was made under UV light, at 254 nm, using a Camag Universal UV Lamp 254, 366 nm (Muttenz, Schweiz).

2.2. UV-VIS spectral studies

UV-VIS spectra were recorded on a Beckman DU 640 spectrophotometer, in 1 cm quartz cuvette, using 0.04 mg ml^{-1} methanol solutions. For quantitative assays, the samples were prepared by appropriate dilution (0.008; 0.010; 0.012; 0.014; 0.016 mg ml^{-1}) with methanol, of the corresponding stock-solutions (0.10 mg ml^{-1}, in methanol). The sample absorbance was recorded at 322 and 402 nm for (**I**), and 255 and 406 nm for (**II**), respectively.

2.3. IR spectral studies

IR spectra were recorded on a Specord M80 spectrophotometer (Carl Zeiss Jena), in potassium bromide pellets (0.1% w/w, substance in KBr).

3. Results and discussions

3.1. TLC studies

Using (A) and (B) solvent systems, we were able to separate and characterise the thin layer chromatographic behaviour of (**I**) and (**II**). The Rf values are summarised in Table 1.

The Rf values for both solvents (A and B) are close related, but solvent A, with hydrophilic properties, leads to a better separation and might be use for identification of these two compounds (**I**) and (**II**) from mixtures.

Table 1
Rf values (run distance of sample/run distance of solvent front) for (**I**) and (**II**), using solvent systems A [acetonitrile-water-formic acid (25:4:2, v/v/v)] and B [benzene-acetonitrile (3:1, v/v)][a].

Compound	Rf value (Solvent system A)	Rf value (Solvent system B)
I	0.807	0.886
II	0.835	0.875

[a] 10 ml of sample, 0.04 mg/ml in methanol, were applied on start line, run time: 45 min, visualisation: UV light, at 254 nm.

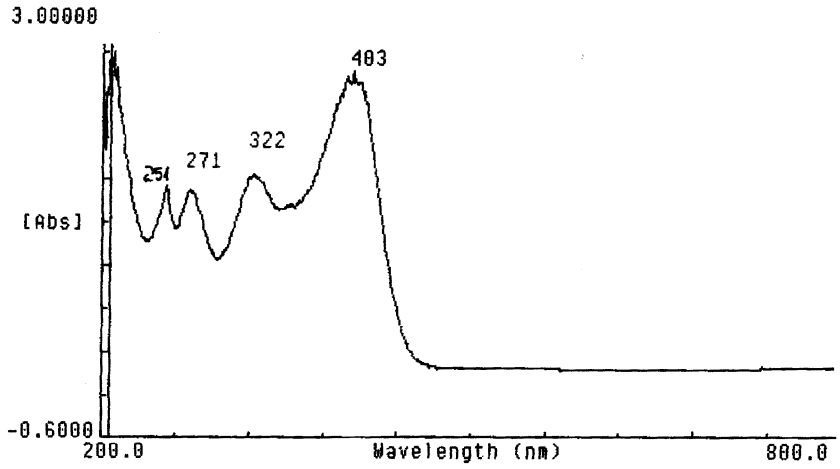

Fig. 2. UV-VIS spectrum for (**I**), 0.04 m ml^{-1}, methanol solution, 1 cm quartz cuvette.

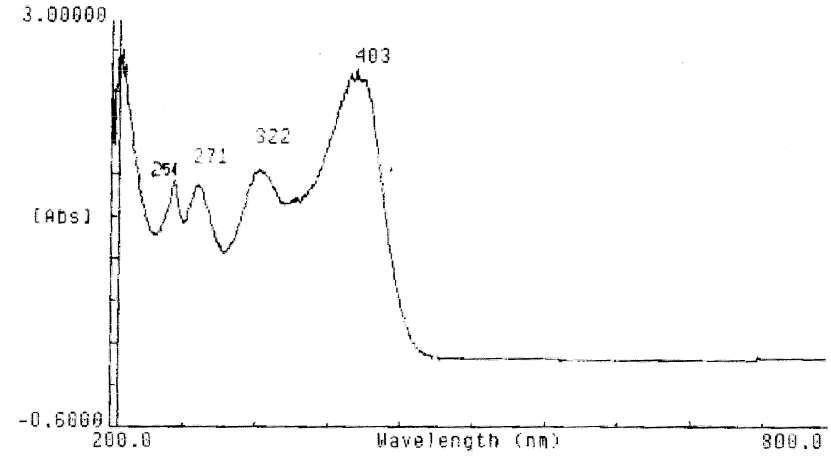

Fig. 3. UV-VIS spectrum for (**II**), 0.04 mg ml^{-1}, methanol solution, 1 cm quartz cuvette.

3.2. UV-VIS and IR spectral studies

The UV-VIS spectra of (**I**) and (**II**) are very similar (Figs. 2 and 3). Compared to (**I**), compound (**II**) has a batochrom effect, due to one more bromine atom. The (**II**) UV-VIS absorbance is slightly diminished, this might be explained considering that we used identical % w/v concentrations, instead molar concentrations. (**II**) has a molecular weight ($M_w = 410.06$), higher than (**I**) ($M_w = 331.64$), thus a smaller number of molecules are available for light absorption. However, the UV-VIS spectra for (**I**) and (**II**) are also similar to other flavone derivatives, the bromine atom(s) having a small influence on spectra profile.

Table 2
Principal IR peaks for (**I**) and (**II**), in KBr pellets

Compound	IR peaks (cm^{-1})
I	1680, 1584, 1408, 1348, 1312, 1264, 1168, 1136, 816, 752
II	1668, 1632, 1584, 1456, 1408, 1312, 1264, 1216, 1168, 764, 624, 592

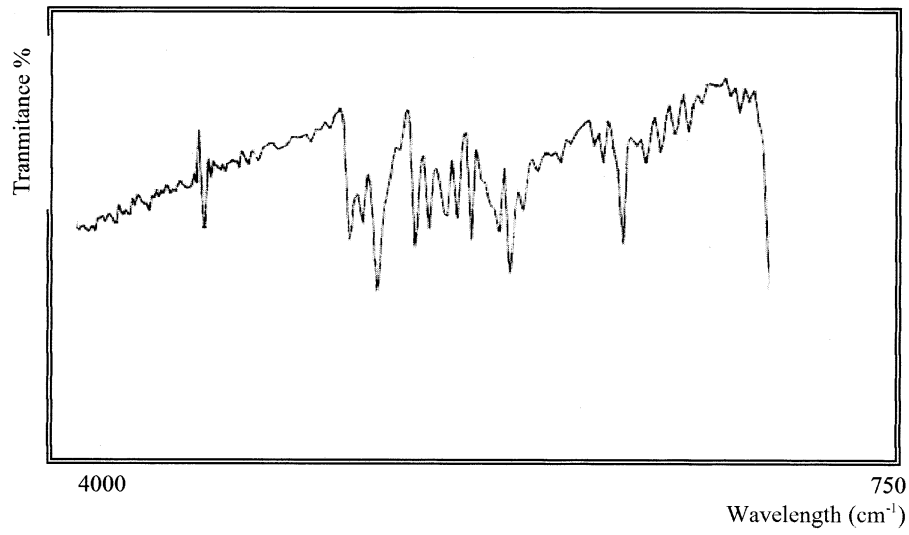

Fig. 4. IR spectrum for (**I**), in KBr pellets. Maximum peaks: 1680, 1584, 1408, 1348, 1312, 1264, 1168, 1136, 816 and 752 cm^{-1}

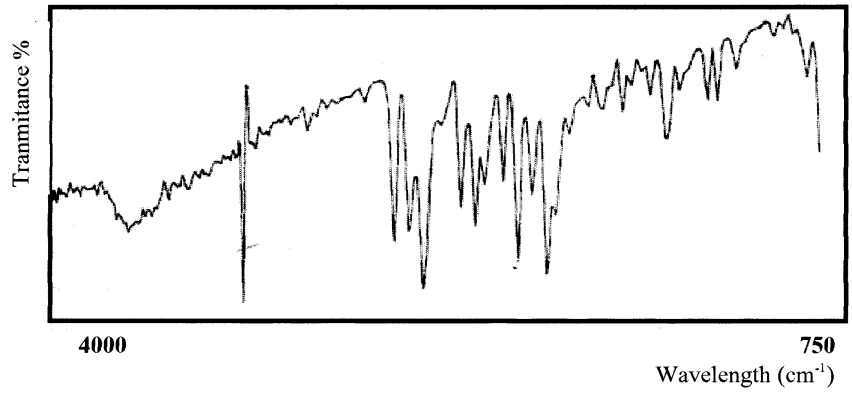

Fig. 5. IR spectrum in KBr pellets for (**II**). Maximum peaks: 1668, 1632, 1584, 1456, 1408, 1312, 1264, 1216, 1168, 764, 624 and 592 cm^{-1}

Table 3
The equations for linear regression for (**I**) and (**II**), at the corresponding wavelengths [322 and 402 nm for (**I**) and 255 and 406 nm for (**II**)], established following the mentioned formulas; A = a•c + b, where A = absorbance, a = slope; c = concentration, b = constant; r^2 = correlation coefficient

Compound	λ (nm)	Equation of linear regression	Coefficient correlation	Slope	Standard error media for slope
I	322 nm	A = 0.428•c + 0	$r^2 = 0.9979$	0.4043	0.0709
	402 nm	A = 0.6455•c + 0.0052	$r^2 = 0.9994$	0.6524	0.0215
II	255 nm	A = 0.251•c − 0.011	$r^2 = 0.9906$	0.2402	0.0085
	406 nm	A = 0.5075•c + 0.00760	$r^2 = 0.9986$	0.5020	0.0134

Table 4
Absorbance values (spectrophotometric method, 1 cm quartz cuvette)

Nr	c (mg ml^{-1})	I		II	
		A ($\lambda = 322$ nm)	A ($\lambda = 402$ nm)	A ($\lambda = 255$ nm)	A ($\lambda =$ 406nm)
1	0.8	0.338 ± 0.020	0.518 ± 0.017	0.191 ± 0.008	0.412 ± 0.011
2	1.0	0.437 ± 0.020	0.656 ± 0.026	0.232 ± 0.07	0.511 ± 0.024
3	1.2	0.512 ± 0.015	0.782 ± 0.030	0.295 ± 0.008	0.623 ± 0.019
4	1.4	0.593 ± 0.025	0.903 ± 0.040	0.350 ± 0.019	0.724 ± 0.044
5	1.6	0.688 ± 0.028	1.040 ± 0.048	$0\ 383 \pm 0.023$	0.813 ± 0.023

The IR peaks for (**I**) and (**II**) are presented in Table 2 and the IR spectra are presented in Figs. 4 and 5.

3.3. Quantitative assay

The curves absorbance vs. concentration were plotted for all maximum peaks from UV-VIS spectra, for both compounds. For each compound, just one peak from UV spectra was chosen [322 nm for (**I**), and 255 nm for (**II**)], and the linearity range was then established. In this range, five concentration values were selected (0.008, 0.010, 0.012, 0.014 and 0.016 mg ml^{-1} in methanol), and were further used to calculate the equations of linear regression. In Table 3 are presented the equations of linear regression for all assays, the correlation coefficient, the slope and the standard error media for slope.

In Table 4 are presented the absorbance values (mean $\pm$ SEM, $n = 6$) for those five selected concentration.

4. Conclusions

We had characterised two new bromo-flavone derivatives, by means of TLC and UV-VIS-IR spectral studies. The obtained results from our study may be used for their identification, together with the already known methods. Two UV and VIS quantitative assay methods were established, based on linear relation between absorbance and concentration. The method's parameters and validations were determined.

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