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SPECTROCOLOURIMETRIC DETERMINATION OF FURANOCHROMONES AND FURANOCOUMARINS IN PHARMACEUTICAL PREPARATIONS

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ABSTRACT

A colourimetric method based on the reaction of khellin, visnagin and ammidin (imperatorin) with aromatic aldhydes in acid media was developed. The colour obtained was used for the determination of ammidin and furanochromones in pharmaceutical preparations. The optimum conditions for colour production and stability were determined, Beer's Lambert's law was obeyed over the concentration range of 4-10 µcg/ml of each of khellin and visnagin and 4-60 µcg/ml for ammidin. The proposed method compared favourably with other reported methods.

INTRODUCTION

Ammi visnaga grows wildly in the Middle East. It is one of the most valuable medicinal plants growing in Egypt due to the pharmacological importance of its furanochromone content mainly khellin and visnagin. Khellin, the most important furanochromone in *Ammi visnaga*, was found to have a relaxing effect on all smooth muscles[1]. It is a potent coronary vasodilator[2] and thus is used for the treatment of coronary insufficiency and angina pectoris[3]. Visnagin, however, has a weaker spasmolytic action on smooth muscles[4]. Moreover, khellin has been recently reported to be more effective and safer in the treatment of leukodermia, than furanocoumarins, especially in resistant cases[5].

Ammi majus also enjoys a good reputation due to the effect of its furanocoumarins, particularly ammoidin and ammidin in the treatment of leukodermia[6].

The reported quantitative analytical methods for khellin and visnagin include either colourimetric[7-9], polarographic[10], gravimetric[11], spectrophotometric[12], I.R. spectrophotometric[13], fluorimetric[14] and HPLC[15] methods. Occasionally chromatographic purification (Column, TLC or paper chromatography) was performed prior to the application of analysis.

Most of the existing methods for the estimation of furanocoumarins are either gravimetric[16], colourimetric using various colour reagents[17-24], spectrophotometric following PC or TLC separation[8,25], densitometric[26], spectrofluorimetric[27] and polarographic[28]. In addition, GLC[29] and HPLC[30] methods were reported.

EXPERIMENTAL

A. Materials:

1. Pure authentic samples of khellin, visnagin, bergapten, ammoidin (xanthotoxin) and ammidin (imperatorin) were kindly donated by the Memphis Chemical Company, Cairo, Egypt.
2. Analytical reagent grades of p-dimethylaminobenzaldehyde, vanillin, anisaldehyde, ethanol and sulfuric acid.

3. Various dosage forms were purchased from the local market.
4. Spectroplus spectrophotometer, M.S.E.

B. Reagents:

1. Alcoholic solutions of khellin, visnagin, bergapten, ammoidin and ammidin (0.1% w/v); 10 ml aliquots were prepared by separately dissolving 10 mg of khellin, visnagin, bergapten, ammoidin and ammidin in 10 ml ethanol.
2. Alcoholic solutions (1% w/v) of the aromatic aldehydes p-dimethylaminobenzaldehyde, vanillin and anisaldehyde.
3. 50% v/v aqueous solution of H_2SO_4.

C. Colour reaction:

An aliquot corresponding to 0.1 mg of each of khellin, visnagin, bergapten, ammoidin and ammidin was separately eveporated on a water bath and the residue was dissolved in sulphuric acid, then the aromatic aldehyde solution was added. The reaction mixture, developed a colour that depended on the aromatic aldehyde used (Table 2).

D. Assay procedure:

An aliquot of 0.1 ml of the alcoholic solution of each of the standard samples under investigation (1 mg/ml) was transferred to a test tube. The solvent was evaporated to dryness on a boiling water bath, the residue was dissolved in 0.2 ml H_2SO_4 and the solution was set aside for 5 minutes and then 0.2 ml of the aromatic aldehyde reagent was added while cooling under tap water to guard against the possible thermal degradation. The mixture was separately diluted to 5 ml of dioxane in the case of furanochromones and 50% sulfuric acid to 5 ml also in the case of furanocoumarins. The absorbance of the resulting coloured liquid was measured after 10 minutes of dilution at a suitable λ_{max} using a blank similarly treated but with neither furanochromones nor furocoumarins (Table 2). The procedure was repeated around the year in different seasons and was found to be stable.

E. Determination of khellin and ammidin in pharmaceutical dosage forms:

The previously mentioned general procedure was applied to determine the khellin and ammidin content in tablets, injections, suppositories, paints, syrups and drops. The procedure adopted for sample preparation before analysis varied according to the nature of the product tested.

1. Tablets containing:

A. Furocoumarins:

A weight, corresponding to 50 mg of ammidin from the pooled content of the powdered tablets was extracted with chloroform. The chloroformic extract was evaporated to dryness and the residue was dissolved in ethanol and the volume made up to 50 ml with ethanol. A volume of 0.1 ml of the solution was used for the assay.

B. Furanochromones:

A weight, corresponding to 100 mg of khellin from the pooled content of the powdered tablets was extracted with chloroform. Since many of the products contained combined alkaloids, the chloroformic extract was shaken with 10 ml of 0.1 N H_2SO_4. The organic phase was separated and washed with 2 X 30 ml aliquots of distilled water, filtered over anhydrous sodium sulphate then evaporated to dryness. The residue was dissolved in ethanol and the volume made up to 100 ml with ethanol. A volume of 0.1 ml of the solution was used for the assay.

2. Injections:

An aliquot, corresponding to 150 mg of khellin, was acidified with 10 ml 0.1 N H_2SO_4. The solution was extracted with chloroform (3 X 20 ml). The combined chloroformic extract was washed with 2 X 30 ml aliquots of distilled water and dehydrated with anhydrous sodium sulphate and the filtrate was evaporated to dryness. The residue was dissolved in 100 ml ethanol and 0.1 ml of solution was used for the assay.

3. Suppositories:

A weight, corresponding to 100 mg of khellin from the pooled suppositories, was melted by adding 50 ml of boiling distilled water. The mixture was cooled and defatted with petroleum ether (60-80 °C). The defatted aqueous solution was acidified with 10 ml 0.1 N H_2SO_4 and extracted with chloroform (3 X 20 ml). The combined chloroformic extract was washed with distilled water (2 X 30 ml) and dehydrated by anhydrous sodium sulphate. The filtrate was evaporated to dryness. The residue was dissolved in 100 ml ethanol and 0.1 ml of solution was used for the assay.

4. Paints:

An aliquot, corresponding to 50 mg of furanocoumarins, was diluted with ethanol to 50 ml and 0.1 ml of the solution was used for the assay.

5. Syrups:

The sample was treated in the same manner as mantioned under the injections.

6. Drops:

An aliquot, corresponding to 100 mg of khellin was evaporated to dryness. The residue was dissolved in 100 ml ethanol and 0.1 ml of the solution was used for the assay.

RESULTS AND DISCUSSION

Furanocoumarins and furanochromones contain a methyl group that can interact with aromatic aldehydes in the presence of sulphuric acid. The colour of the reaction mixtures and its maximal absorbance differed according to the specific aromatic aldehyde used.

From Table 2, it is clear that both p-dimethylaminobenzaldehyde and anisaldehyde were suitable for the determination of khellin and visnagin since they produced a stable colour, while vanillin was not suitable for the purpose since it produced a colour which was unstable and fades with time with both compounds.

In the case of furanocoumarins, only ammidin could be determined by the reaction of either vanillin or anisaldehyde since they gave a stable violet colour after 10 minutes of dilution, while both ammoidin and bergapten gave a yellow colour that faded with time. Therefore the method could be used for the determination of ammidin in a mixture with ammoidin and/or bergapten without prior chromatographic separation. The estimation of the total furanocoumarins could be performed using other reported methods[8,24].

The developed colours were stable after 10 minutes for at least 3 hrs as was confirmed by the unchanged spectrophotometric absorbance of the reaction mixtures of standard furanocoumarins and furanochromones during that period. The colour was stable at different seasons i.e. the variation of room temperature had no effect on the stability of the colour. Dioxane proved to be the most suitable solvent for

the estimation of khellin and visnagin, while 50% H_2SO_4 was suitable for ammidin. A volume of 0.2 ml of the alcoholic solution of the aromatic aldehyde proved to provide a satisfactory colour reaction. Beer's Lambert's law was valid over the concentration range of 4-100 μg/ml for each of khellin and visnagin and 4-60 μg/ml for ammidin.

Table 2 clearly shows that the proposed method compared well with the reported methods[23-24] for furanochromones and furocoumarins determination. Recovery determination proved that the method is accurate within ± 2% (Table 3). The method is quite suitable for the determination of khellin and ammidin in their dosage forms and in this regard compares well with other reported methods. Furthermore, the pharmaceutical adjuncts of the doses tested did not interfere with the analysis of the proposed method. The weakly basic alkaloids papaverine and theophylline, present in some dosage forms with khellin, although are extracted with khellin in the acidic chloroform, did not interfere with the colour formation i.e. they themselves do not give a colour with the aromatic aldehydes used. In the case of suppositories, defatting with petroleum ether did not remove any khellin as tested by TLC. This was confirmed by partitioning the pet. ether extract between acetonitrile and petroleum ether, evaporating the acetonitrile fraction and testing the residue by TLC. The acetonitrile fraction was free of khellin. The method, however, could not be used for the analysis of extracts of *A. visnaga* and *A. majus*, since the pigments and other constituents in the extracts could interfere with the assay.

Table (1): Colour reactions of furanochromones and furocoumarins with the different aromatic aldehydes.

Aromatic aldehyde	Compound tested	Colour	λ_{max}	Stability of the colour produced
p-dimethylamino-benzaldehyde	Khellin	Yellow	400 nm.	Suitable (colour stable with time)
	Visnagin	Yellow	300 nm.	Suitable (colour stable with time)
	Ammidin	Yellow	---	Unstable (colour fades upon dilution)
	Ammoidin	Yellow	---	Unstable (colour fades upon dilution)
	Bergaptin	Yellow	---	Unstable (colour fades upon dilution)
Anisaldehyde	Khellin	Yellow	405 nm.	Suitable (colour stable with time)
	Visnagin	Yellow	380 nm.	Suitable (colour stable with time)
	Ammidin	Violte	524 nm.	Suitable (colour stable with time)
	Ammoidin	Yellow	374 nm.	Unstable (colour fades upon dilution)
	Bergaptin	Yellow	368 nm.	Unstable (colour fades upon dilution)
Vanillin	Khellin	Yellowish green	404 nm.	Unstable (colour fades upon dilution)
	Visnagin	Yellowish green	390 nm.	Unstable (colour fades upon dilution)
	Ammidin	Violet	540 nm.	Suitable (colour stable with time)
	Ammoidin	Yellow	380 nm.	Unstable (colour fades upon dilution)
	Bergaptin	Yellow	370 nm.	Unstable (colour fades upon dilution)

Table 2: Determination of khellin and ammidin in pharmaceutical dosage forms.

Products	Trade name	Ingredients	Labeled amount	Found amount and %*	Reported method***
		Furocoumarins*		**	
Tablets	Meladinine	Ammoidin Ammidin	10 mg/tab. 5 mg/tab.	5.04 mg/tab. (90.0%)	102.03%
Paint	Meladinine	Ammoidin Ammidin	7.5 mg/tab. 2.5 mg/tab.	** 2.47 mg/ml (100.77)	101.9%
		Furanochromones+			
Tablets	Lynamine Compound	Khellin + Papaverine HCl	100 mg/tab.	102.7 mg/tab. (102.7%)	102.4%
	Lynamine	Khellin	20 mg/tab.	20.58 mg/tab. (102.9%)	102.3%
Injections	Khellalgine	Khellin + atropine HSO_4	50 mg/5 ml	51.5 mg/5 ml (103.0%)	102.7%
	Glucolynamine	Khellin +	30 mg/10 ml	30.8 mg/10 ml (102.6%)	103.1%
	Glucolynamine compound	Khellin + theophylline + papaverine HCl	30 mg/10 ml	29.5 mg/10 ml (98.3%)	99.1%
Suppositories	Lynabros	Khellin + codeine phosphate	10 mg/supp.	10.33 mg/supp. (102.7%)	103.3%
	Khellalgine	Khellin + atropine HSO_4 + papaverine HCl	50 mg/supp.	51.29 mg/supp. (102.85)	99.0%
Syrup	Lynabros	Khellin + codeine phosphate	200 mg/100 ml	200.74 mg/100 ml (103.1%)	103.7%
Drops	Lynamine	Khellin	20 mg/ml	20.6 mg/ml (100.3%)	101.0%

* = Average of three determinations.

** = The stated method is not suitable for the determination of ammoidin.

*** = According to the method of Moustafa et al.[24].

X = P-dimethylaminobenzaldehyde is used in case of khellin.

\+ = Anisaldehyde is used for ammidin.

Table 3: Determination of percentage recovery.

Products	Trade name	Ingredients	Enrichment amount of the comp. added	Found amount mg/ml)* before enrich.	Found amount mg/ml)* after enrich.	Recovery
Tablets		Furocoumarins[X]				
	Meladinine	Ammoidin	**	**	**	**
		Ammidin	1 mg/ml	1.01	2.015	99.7%
Paint	Meladinine	Ammoidin	**	**	**	**
		Ammidin [+]	1 mg/ml	0.99	2.01	102.2%
Tablets		Furanochromones[X]				
	Lynamine CO	Khellin+Papaverine HCl	1 mg/ml	1.02	2.00	98.0%
	Lynamine	Khellin	1 mg/ml	1.03	2.04	100.7%
Injections	Khellalgine	Khellin+atropine 90,	1 mg/ml	1.04	2.03	97.0%
	Glucolynamine	Khellin+theophylline	1 mg/ml	1.03	2.04	101.0%
	Glucolynamine CO	Khellin+theophylline+papaverine HCl	1 mg/ml	0.93	2.00	102.0%
Suppositories	Lynabrom	Khellin+Codeine phosphate	1 mg/ml	1.03	2.03	100.0%
	Khellalgine	Khellin+atropine HSO_4 + papaverine HCl	1 mg/ml	1.02	2.04	102.0%
Syrup	Lynabrom	Khellin+codeine phosphate	1 mg/ml	1.04	2.04	100.3%
Drops	Lynamine	Khellin	1 mg/ml	1.03	2.03	97.6%

* Average of three determinations.

** The stated method is not suitable for the determination of ammoidin.

X P-dimethylaminobenzaldehyde is used in case of khellin.

\+ Anisaldehyde is used for ammidin.

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طريقة طيفية للتقدير الكمى للفيورانوكرومونات والفيورانوكومارينات فى المستحضرات الصيدلية

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تم أستنباط طريقة لونية لتقييم كل من الخلين والفزناجين والاميدين تعتمد على تفاعل تلك المواد مع المواد الالدهيدية الاروماتيكية وهى (بارادايميثيل أمينوبنزالدهيد ، انيسالدهيد والفانلين) وذلك فى وجود حمض الكبريتيك وقد أمكن استخدام هذه الطريقة لتقدير كميات الخلين والاميدين فى المستحضرات الصيدلية وقورنت الطريقة بالطرق الاخرى المنشورة. وأمكن تقدير كمية الاميدين بهذه الطريقة فى وجود كل من الاموبدين والبرجابتين بدون فصل مسبق باستخدام الفانلين أو الانيسالدهيد حيث أن الاميدين يعطى لونا بنفسجيا ثابتا بينما يعطى كل من الاموبدين والبرجابتين لون أصفر يضعف مع مرور الوقت والطريقة حساسة ووجد أن نسبة الاسترجاع عالية بزيادة أو نقص ٢٪ ويمكن أستخدام الطريقة المذكورة فى تقدير خلاصات الخلة البلدى والخلة البرى بعد التنقية كروماتوجرافيا لازالة الصبغات الموجودة فى الخلاصات.

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