Simultaneous Determination of Main Effective Constituents for Hops Extracts

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Abstract

Humulus Lupulus L. (Hop) is a dioecious and perennial plant of Cannabaceae that important medical and industrial plant in the world , which has traditional uses in treatment insomnia ,restlessness , nervous tensions , irritability , charleyhorse , etc. The reportage antibacterial and antifungal effects and potentially giving them anticancer of plant .

There is a method for determining of alfa and beta-acids for medicinal uses of hops extracts in pharmacopeias such as BP and USP, but it takes more times and has low precision and accuracy in analysis data. Thus it is useless for many industries. 2-Methyl-3-buten-2-ol, myrcene and alfa-humulene are three main constituents in essential oil part of Hops extracts were determined in the extract directly as simultaneous analysis.

The method was based on internal standard by Gas chromatography. Determined amount of the extract was shaking with determined amount of dichloromethane and after separation of the dichloromethanic phase, the internal standard was added and injected to a temperature programmed GC. Concentration of main fractions like as 2-Methyl-3-buten-2-ol, myrcene and alfa-humulene were determined by internal standard method and with calibration curve of each substance. The method was validated and used for comparing some Iranian and other countries Hops extracts. This study revealed that the concentration of 2-methyl-3-buten-2-ol, myrcene and alfa-humulene in Hops extract was 12.6, 30.6 and 2.5 mg/100ml respectively , also considering validation percentage of precision 2.8%, 3.9% and 29.9% respectively .

***Keywords*:** Humulus lupulus, 2-methyl-3-buten-2-ol, myrcene , alfa-humulene, GC, internal standard method

Introduction

Humulus Lupulus L. (Hop) is a member of the Cannabaceae family , native to Europe, North America and parts of Asia . The plant is also valued for its astringent and antibiotic properties and traditional uses in treatment insomnia , restlessness , nervous tensions , irritability , charleyhorse , etc [(1)]. Also in modern medicine is antibacterial and antifungal and potentially giving them anticancer and uses for relaxation [(2)].The medicinal parts are the glandular hairs separated from the infructescence, the whole dried female flowers, the fresh or dried female inflorescence [(3)].The male flowers are yellowish-greenish,

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inconspicuous and about 5 mm in diameter. The female flowers are in richly blossomed, heavily branched inflorescence [4]. The ovary, which has 2 long, downy stigmas, is surrounded at the base by round compressed nutlet. A yellowish fruit cone grows from the female flower. The inside of the bracts is covered with small, glossy, light yellow glandular scales, which contain hop bitter (Lupulin).The hop plant is a perennial. The annual shoots reach a height of 6 (12m when cultivated). The stems are pencil-thick, green and do not turn woody. They are covered in 6 rows of climbing barbs. The leaves are 3 to 5 lobed, serrate, and opposite [4]. Hop cones consist of the whole dried female inflorescence of *humulus lupulus*. After the harvest, the hops are dried on racks at 30 to 60 ^{0}C [5]. The plant constituents are alpha-bitter acids including, among others, lupulone, colupulone and adlupulone. Volatile oil (0.3 – 1.0%) very complex in makeup, chief components myrcene, humulene, beta-caryophyllene and undecane-2-on, furthermore 2-methyl-3-buten-2-ol (particulary following storage , as breakdown product of the acylphloroglucinols). resins(oxidation products of the bitter acids) [3], phenolic acid, including among others, ferlic acid, caffeic acid and their derivatives,for example chlorogenic acid , tannins, oligomeric proanthocyanidines, flavonoids, including ,among others, xanthohumole [6].

Medicinal Effects

Neurosedative, antibacterial, antifungal, diuretic, antitumor, and sterogenic activities have been stablished. Many of effect are thought to be due to the volatile oil (2-methyl-3-buten-2-ol), flavonoids, and/or estrogenic activity of the plant [4,7,8,9,10]. Hops is primarily used for its mild sedative effects and for treating the gastrointestinal system [11]. Hops in combination with valerian, balm leaf, and motherwort produced sedative effects in one human study. This effect however, strongly depends on the quality of the extract used [11]. Hops is a bitter, an astringent, and a smooth-muscle relaxant with antibacterial properties. This makes it useful in the treatment of indigestion, nervous gastropathies, colitis, and irritable bowel syndrome as well as preventive from individuals prone to ulcers [10].

Research importance

Hops extracts are very used in medicinal products as told above. For this reason many producers interest to control extracts quality and quantity. Tow important objects were followed in this research; obtaining a new, standard, valid and simple method for simultaneous determination of main effective of hops extracts.And comparing the species of hops cultivated in Iran (special in Golestan province - North eastern of Iran) with other hops extracts. Although there is a method for determining of alfa and beta-acids (total bitter acids) for medicinal uses of hops extracts in pharmacopeias (such as BP and USP methods) [3], but it takes so much times and has low precision and accuracy in analysis data. Thus it is useless for many industries and QC units. Since main medicinal effects of hops extracts are in volatile oil part, essential oil analysis could be a best method for quantity control. Three main constituents in essential oil part of Hops extracts that have more medicinal value are 2-methyl-3-buten-2-ol, myrcene and alfa-humulene which determined in the extract directly as simultaneous analysis.

Materials and Methods

Determination was based on an internal standard method by Gas chromatography. The method was home method and need to valid. Therefore procedure of analysis was as follow.

Sample Preparation

1) 25 ml Dichloromethane was added to 15ml sample extract in a 120ml vial and after sodium chloride addition (for salting out) it was shaken for 30 minutes.

2) The solution was left about 10 minutes in a decanter to separate organic and inorganic phase
3) Dichloromethanic phase was collected by a paper filter in a 50 ml graduate cylinder and added dichloromethane to 30 ml volume.
4) 8ml Of the final solution was mixed homogenately with 2 ml standard decanal solution 1000 ppm (as internal standard) in a 10 ml volumetric flask.
5) 4 Micro liter of prepared solution was injected in a Gas chromatography instrument temperature programmed as below
6) 2-Methyl-3-buten-2-ol, myrcene and alfa-humulene was determined by each calibration curve that obtained as follow.

Instrumental conditions: GC- VARIAN CP-3800
Column: WCOT fused silica DB-wax 52 CB- 50m length - thickness 0.2µm
Programming: 60 ^{O}C Hold 9min.-60 ^{O}C to 240 ^{O}C (10^{O}C per min.),Total 27 Min.
Injector: 200 ^{0}C- FID 290 ^{0}C

Standard Solutions

1) All standard solutions were made from their Merck standards (2-methyl-3-buten-2-ol, myrcene and alfa-humulene). A 10000 ppm stock solution from each standard was made and five secondary standards with 50, 100, 200, 300, 400 and 500 ppm concentration were prepared in a 10 ml volumetric flask for each solution. 2 ml of 1000 ppm decanal solution (internal standard) was added to each flask and diluted with was ethyl alcohol 96%.

2) 4 Micro-liter of each fresh prepared standard was injected in GC as above conditions at least twice.
3) For recovery coefficient determination a 100 ppm standard solution for each compound was extracted with dichloromethane as told in past part and injected in GC twice.

From calibration curve and recovery coefficient three compounds (2-methyl-3-buten-2-ol, myrcene and alfa-humulene) of the sample extract were determined.

Results and Discussion

Area injection of all solutions (samples and standards) were calculated by the instrument's software. Ratio of the samples are injection to internal standard area injection are shown in Table 1.

Calibration curve for three main constituents, 2-methyl-3-buten-2-ol, myrcene and alfa-humulene, of the extract are shown in figure 1, 2 and 3 respectively**.**

Discussion

As it is shown in above we can determine quantity of three main constituents of Hops extracts simultaneously. But amount of precise and accuracy in our home method is logically accepted. By the way in some classic method as BP and USP method [(3)], it is impossible that we determine the extract constituents. And we could not determine a real concentration of constituents (even bitter acids) [(4)]. Also in our home and new method, control of the extracts, can be directly done on extracts and it is a preference over other methods to determine an effective constituents of an extract simultaneously directly, rapidly, precisely and validly. Finally it is non-denial.

Name	Conc. (µg/ml)	Area Ratio inj-1 for But-2-ol	Area Ratio inj-2 for But-2-ol	Ave.For But-2-ol	Area Ratio inj-1for Mcn.	Area Ratio inj-2 for Mcn.	Ave.For Mcn.	Area Ratio inj-1 for Hln.	Area Ratio inj-2 for Hln.	Ave.ForHln
Std. Solution of: Meth. But-2-ol, Myrcen, α-Humulene	*50ppm*	0.41	0.30	***0.36***	0.39	0.27	***0.33***	0.18	0.18	***0.18***
Std. Solution of: Meth. But-2-ol, Myrcen, α-Humulene	*100ppm*	0.59	0.56	***0.57***	0.64	0.60	***0.62***	0.36	0.37	***0.36***
Std. Solution of: Meth. But-2-ol, Myrcen, α-Humulene	*150ppm*	0.85	0.83	***0.84***	0.94	0.94	***0.94***	0.56	0.54	***0.55***
Std. Solution of: Meth. But-2-ol, Myrcen, α-Humulene	*200ppm*	1.04	1.05	***1.04***	1.13	1.15	***1.14***	0.72	0.73	***0.72***
Std. Solution of: Meth. But-2-ol, Myrcen, α-Humulene	*300ppm*	1.57	1.75	***1.66***	1.78	1.96	***1.87***	1.09	1.11	***1.10***
Std. Solution of: Meth. But-2-ol, Myrcen, α-Humulene	*400ppm*	2.22	2.19	***2.20***	2.53	2.48	***2.5***	1.47	1.48	***1.47***
Recovery for Std. Solution of: Meth. But-2-ol, Myrcen, α-Humulene	*200ppm*	0.5	0.51	***0.5***	0.43	0.43	***0.43***	0.25	0.22	***0.24***
Hops Extract	-	0.31	0.31	**0.31**	0.68	0.61	**0.65**	0.04	0.05	**0.045**

Table 1. Samples are to internal standard area injection ratio

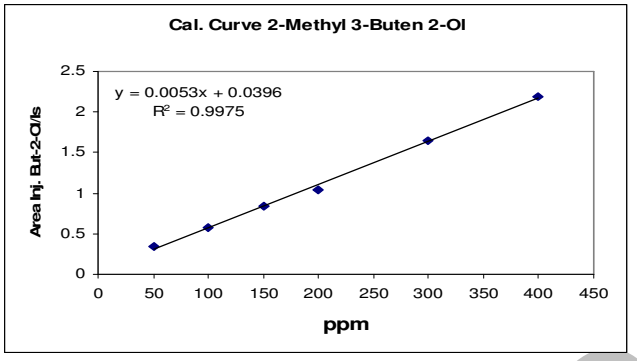

Fig. 1. Calibration curve for 2-Methyl-3-buten-2-ol

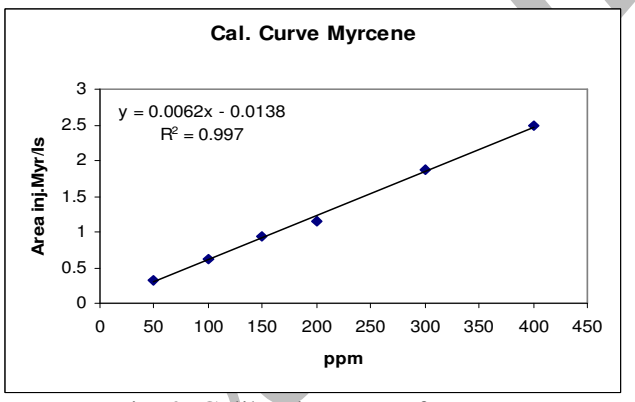

Fig. 2. Calibration curve for Myrcene

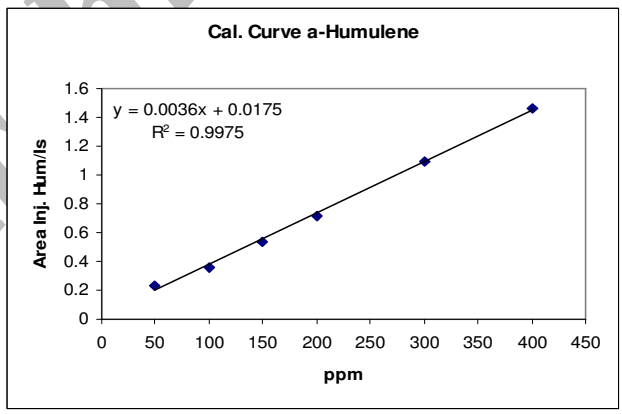

Fig. 3. Calibration curve for alfa-Humulene

Due to calibration curves and Table 1, recovery coefficient for three constituents were calculated as follow:

Recovery for 2-Methyl-3-Butene-2-ol
(0.5- 0.0396)/0.0053 = 86.8 ppm Dilution: 86.8 *(30/15)*(10/8)=217.2ppm
Recovery Coefficient: (217.2/ 200) *100= **100.8%**

Recovery for Myrcene
(0.43-0.0138)/0.0062 = 67.1 ppm Dilution: 67.1 *(30/15)*(10/8)=167.8 ppm
Recovery Coefficient: (167.8/ 200) *100= **83.9%**
Recovery for α-Humulene
(0.24-0.0175)/0.0036 = 61.8 ppm Dilution: 61.8 *(30/15)*(10/8)=154.5 ppm
Recovery Coefficient: (154.5/ 200) *100= **77.3%**

And finally concentration for each compounds were obtained:

2-Methyl-3-Butene-2-ol in H-851 : (0.31-0.0396)/0.0053=51.02 ppm
with Dilution and recovery: 51.02 *(30/15)*(10/8) * (100/100.8)=126.5 ppm= **12.6mg/100ml**

Myrcen in H-851 : (0.65-0.0138)/0.0062 = 102.6 ppm
with Dilution and recovery: 102.6 *(30/15)*(10/8) * (100/83.9)=305.7 ppm= **30.6 mg/100ml**

α-Humulene in H-851: (0.045-0.0175)/0.0036 = 7.64 ppm
with Dilution and recovery: 7.64 *(30/15)*(10/8) * (100/77.3)=24.6 ppm= **2.5 mg/100ml**

Validation control

Since the method used in the research is home method, it needs to be valid by statistical methods. Each analysis was performed for 10 times to obtain a suitable quantity of data analysis. Table 2 shows this data.

No of Injection	Area Ratio for 2-Methyl-3-But-2-o l /Int.Std	Area Ratio for Myrcene / Int.Std	Area Ratio for Alfa Humulene / Int.Std
1	0.31	0.68	0.04
2	0.31	0.61	0.05
3	0.32	0.64	0.05
4	0.33	0.65	0.04
5	0.34	0.60	0.07
6	0.31	0.59	0.07
7	0.32	0.59	0.09
8	0.34	0.61	0.11
9	0.31	0.58	0.11
10	0.32	0.59	0.08

Table 2. Necessary data for 10 times injection

Averages of the ratios are:

Area Ratio for 2-Methyl-3-But-$\overline{2}$-o l /Int.Std- $X=\sum X_i/n = 0.32$
Area Ratio for Myrcene /Int.$\overline{S}$td $X=\sum X_i/n = 0.61$
Area Ratio for Alfa-Humulene $\overline{\ }$/Int.Std $X=\sum X_i/n = 0.07$

Precise for these analysis are:

2-Methyl-3-But-2-o l $P\%=[\sum (|\overline{X}-X_i|/\overline{X})/n]100 = 2.8\%$

Myrcene $P\%=[\sum (|\overline{X}-X_i|/\overline{X})/n]100 = 3.9\%$

Alfa-Humulene $P\%=[\sum (|\overline{X}-X_i|/\overline{X})/n]100 = 29.9\%$

Conclusion

The study has shown that simultaneous determination of three main effective constituents for Hops extracts is possible with a standard and validated instrumental method. The concentration of 2-methyl-3-buten-2-ol, myrcene and alfa-humulene in Hops extract was 12.6, 30.6 and 2.5 mg/100ml respectively, also considering validation percentage of precision 2.8%, 3.9% and 29.9% respectively .

References:

1. Luppold, E., *Pharmazie in unserer Zeit.*, **13**, 65 (1984).
2. Della-Loggia, R., Medaelli, C., Tobaro, A., and Zillic, C., *Plant Med.*, **51**, 359 (1984)
3. Hartke, K. and Mutschler,E., *DAB9 kommentar, Stuttgart: Wissenschaftliche Verlagsgesellschaftmb H*, 2061 (1987)
4. Salvador, R.L., *Journal of AOAC Methods*, *Hops*, 48 (1994)
5. Widmaier, W., *Pflanzenheilkunde*, WBV Verlag, Schorndorf (1986)
6. Amanlou, M., Farsam, H., and Soleimani, Ataie S., *Iranian Journal of Pharmaceutical Research*, www.ijpr-online.com, 246 (2004).
7. Ngo, E. and Nutter, L., *Biochem Pharmacol*, **55**(4), 505 (1998)
8. Martini M, Seiller M., *Actifs at additifs en cosmetologie*, Technique & Documentation - Lavoiser, Paris (1992).
9. Podlech, D., *Flora Iranica. Akademische Drack U. Verlagsanstalt*, **158**, 80 (1986)
10. Wichtl.M., *Ein,. Handbuch fur die Praxis auf Wissenscbaftlicher Grundlage,* Wissenschaftliche Verlagsgesellschaftmb H, Germany, 263 (1989)
11. Pasechnik, I.K., *Farmacol. Toksikol*, **27**, 486 (1986)