International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Qualitative Analysis of Ayurvedic and Allopathic Cough Medicines within Haryana

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Abstract: ***Cough Syrups and tablets are some of the most obtained OTC (Over the Counter) Drugs by people all over India. Also, during the times of COVID-19, an increase in the purchase of such medicines was noted. These medicines are commercially available under different brand names, although their components may be closely related. Qualitative analysis can help in the identification of such compounds and also compare them with that of the existing ones. This can be of use in cases of adulteration, death due to overdose, drug abuse and also in the quality management of such manufacturing agencies. In the present study, qualitative analysis of some commercially available allopathic and ayurvedic medicine using Thin Layer Chromatography has been conducted. The samples were subjected to a step-by-step process for preparation prior to spotting. The solvent system of choice comprised chloroform: methanol in the ratio of 90: 10 (v/v). The TLC plates were visualized under UV light to spot the distances travelled by the solute and solvent respectively. The Rf values calculated were then used to identify the components.***

Keywords: Over the Counter, Qualitative Analysis, Thin Layer Chromatography, Adulteration, Abuse

1. Introduction

Any substance other than food that modifies the physiological system and is used for preventing, curing, or relieving the symptoms of any abnormal condition of the body is defined as a drug. Medicines are commercially manufactured under proper quality assurance to make them effective against specific diseases and be harmless to the consumer simultaneously. Medicines which are extracted from nature and used aur called ayurvedic medicines and medicines which are processed in factories and then used are called allopathic medicines. Over the counter medicines are the ones that are available without any prescription of doctor, and they are most commonly used for self-medication. These drugs are directly purchased from any pharmacy outlet. The most commonly purchased OTC drugs are cough-cold preparations, antacids, analgesics and so on. There are more than 20, 000 prescribed drugs and almost 3, 50, 000 over the counter drugs so it is really important to know all the adverse effects of the medicines and rationalize their use[1]. Self-medication or without prescription drugs can be harmful and have high chances to be abused. Also, the ease of procurement makes their abuse so prominent. The COVID-19 pandemic was one such scenario where the sale of such OTC medicines had increased. The symptoms of the Covid flu primarily showed cough, cold and fever, thereby causing a huge demand for such medicines during that time. Research has also shown the use of molecular docking to study the phytochemicals and active pharmacological components in Indian medicinal herbs namely Tulsi, Giloy, Clove, Cardamom and Ashwagandha especially in the treatment of the novel Coronavirus[2]. However, even the ayurvedic preparations have been abused over years, one such drug includes “Barshasha” which is an opium containing Unani preparation that is often sold as an OTC drug for common cold[3, 4]. Dextromethorphan is the major component, which is present in allopathic cough syrups, and has potential to be abused. Apart from abuse, the purity of the cough syrups also needs to be regularly monitored for quality assessment. Adulterated cough syrups can cause fatal damage. One such case was reported in Kashmir, where Diethylene Glycol, a toxic industrial solvent was found in the cough syrup whose ingestion led to the death of 12 children. In this study we have used TLC as our analysis technique to examine the presence of components in common ayurvedic and allopathic OTC cough-cold preparations[5]. Thin Layer Chromatography has added advantages like easy availability of equipment, low costs, greater separation of components in a mixture etc. TLC (Thin Layer Chromatography) is a sensitive technique that can be used to analyse microgram (0.000001g) quantities and it is very time efficient technique, it takes about 5-10minutes for its analysis. The principle of TLC is based on adsorption, retention, and capillary action. The compounds in the mobile phase move over the surface of the stationary phase.

The movement occurs in such a way that the compounds which have a higher affinity to the stationary phase move slowly while the other compounds travel fast. TLC can be performed in the sequence of spotting, development, and visualization. Thin Layer Chromatography Plates can either be prepared using Silica Gel G slurry or they can be purchased as ready-made ones. The stationary phase is applied on its surface in the form of a thin layer. The stationary phase on the plate has a fine particle size and also has a uniform thickness. Now the plates are ready for sample spotting. The samples can be spotted using a fine capillary tube[6]. In case of pure samples, 10-15 spots should be taken. After every spot it should be allowed to

air dry to make sure the diameter of the spot does not increase much. The spots can be applied on the Solute front of the TLC Plate. All the samples should be spotted such that they are equidistant from each other. Once the sample has been spotted, the TLC plate can be immersed in the mobile phase, in the solvent chamber. After the samples have risen till the solvent front, the TLC plate is removed and dried. If the separated components are visible with the naked eye, then the distances travelled by each sample is marked. In case if the samples are not visible, certain visualization techniques like Iodine fuming, spray reagents like Ninhydrin and Dragondorff can also be used. Thin Layer Chromatography is used for the qualitative analysis of various medicines for example anticonvulsants, sedatives, histamines, tranquilizers, analgesics, steroids, hypnotics, local anaesthetics. TLC is extremely useful for analysis like biochemical as well, separation or isolation of biochemical metabolites from its blood plasma, urine, body fluids, serum. It is also used in several food industries to identify and separate colours, preservatives and sweetening agents[7].

2. Materials and Methods

Materials:

There are a total 8 samples of which 5 are allopathic and 3 are Ayurvedic. All these preparations have been purchased from the local medical shops near Chandu-Budhera village in Gurugram, Haryana. All the chemicals, filter paper, apparatus and centrifuge have been used from the Departmental laboratory.

Methods of Sample Preparation:

The samples have been prepared as follows:

For tablets: 2 tablets of each medicine were powdered and mixed with the help of pestle and mortar.0.5 mg of each crushed tablet was added to 2 ml mixture of chloroform and methanol (1: 1 v/v) in a test tube and kept for a few minutes. The contents were then filtered through Whatman filter paper no.1. The filtrate was collected and centrifuged at 7000 r. p. m for 10 minutes. The supernatant was then transferred to another clean test tube for TLC analysis.

For syrups: 0.5 ml of syrup was added to 2 ml mixture of chloroform and methanol (1: 1 v/v) and kept for a few minutes. Contents were then filtered through Whatman filter paper no.1. Filtrate was collected and centrifuged at 7000 r. p. m for 10 minutes. The supernatant was then transferred to another clean test tube for TLC analysis.

TLC Plate Preparation and Sample Spotting: Pre-Prepared TLC plates were cut into (10cm x10cm) for spotting. Two lines were drawn horizontally at a distance of 1cm from the top and bottom of the TLC plate. The line on the bottom is called as the solute front and that on the top is called as the solvent front. The samples were spotted on the solute front equidistant to each other. The solvent chamber contained chloroform: methanol in the ratio of 90: 10 (v/v). The solvent chamber was covered with an aluminium foil to retain the moisture. The TLC plate was then kept at an angle of 45^0 in the solvent chamber to allow the separation of the components. The capillary action allows the solvents and sample to rise upward. Once the solvent has reached the solvent front, the TLC plate is removed from the solvent chamber and allowed to dry. The plate once dried was subjected to iodine fuming to visualize the sample spots. The spots were then marked with a pencil.

3. Result

The Retention Factor of the samples was calculated as follows:

Rf = Distance travelled by the solute

Distance travelled by the solvent

Table 1: Rf values of Detected Compounds

S. NO	Sample	Rf Value	Assumed Compound
1	A	0.87	Tulsi
2	B	0.62	Guaifenesin
3	C	0.501	Mulethi
4	D	0.601	Guaifenesin
5	E	0.77	Paracetamol
6	F	0.430	Unknown
7	G	0.79	Paracetamol
8	H	0.76	Tinospora

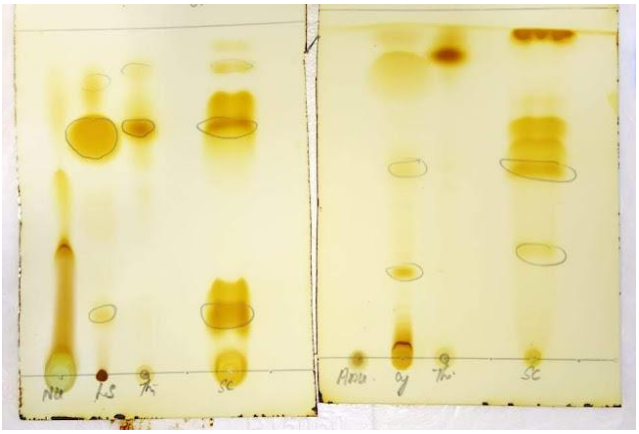

Figure 1: Iodine Fuming to visualize the separated components

4. Discussion

The Rf values of the samples were calculated and compared to identify the components. The first sample "A" showed a Rf value of 0.87. It was considered to be Tulsi (*O. Sanctum*) extract as a similar study by Reddy et al demonstrated the Rf value of flavonoids content in Tulsi leaf extract in a solvent chamber of methanol: chloroform: water (10: 10: 3) to be 0.82[8]. The sample "B" and "D" showed a Rf value of 0.62 and 0.601 respectively. The samples are assumed to be that of Guaifenesin (GFN) as in a study by Jain et al a solvent system of toluene: methanol: ethyl acetate: acetic acid (7: 0.8: 1.2: 0.5 v/v/v/v) gave the Rf value of GFN as 0.6[9]. The sample "C" gave a Rf value of 0.501 and it was

considered to be Mulethi (Liquorice) as studied by Chauhan et al in a solvent system of butyl alcohol, water and glacial acetic acid (7: 2: 1) to be 0.5[10]. The samples "E" and "G" have shown a Rf value of "0.77" and "0.79" respectively and have been assumed to be of Paracetamol. Bebawy et al in their study had shown the Rf value of paracetamol in a solvent system of ethyl acetate: methanol: ammonia 25% (85: 15: 5 v/v) to be 0.77 as well[11]. The sample "H" was found to have a Rf value of 0.76 which corresponded to a study done by Omkar et al where a solvent system comprising Tolune: ethyl acetate: diethylamine 70: 20: 10 gave a Rf value of 0.76 for alkaloids of *Tinospora cordifolia (*Giloy Satva) [12]. The sample "F" had a Rf value of 0.430 which did not give positive identification for any standard compound.

5. Conclusion

Due to the combination of different chemicals in the OTC preparations, the analysis becomes tedious. Thin layer chromatography being a cost efficient and simpler technique helps in obtaining a preliminary identification. High Performance Thin Layer Chromatography has been adherently used as a detection technique for such compounds. The solvent system comprising chloroform and methanol was found to give ideal separation. The Iodine fuming technique was found to be the most appropriate method for visualization. It is non-destructive, faster and accurate responses. With the ever-growing technology, High Performance Thin Layer Chromatography along with other modified forms of column chromatography can give more efficient separation and results. These separation techniques give more ideal results if they are coupled with detection techniques namely spectroscopy.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation**.**

Acknowledgments

The authors would like to thank SGT University, Faculty of Science, Forensic Laboratory for their kind support during all lab studies.

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