$M_t/M_\infty = Kt^n$, where M_t/M_∞ represent fraction of drug released after time t, K a coefficient and n release exponent. The linear regression analysis shown as r values in Table 1 demonstrate that all the five fabricated tablets followed zero-order drug release kinetics. Though commercial tablets A, G, H, I and J also exhibited zero-order drug release kinetics, release rates from them were much faster as compared to fabricated tablets. The n values for fabricated tablets were in the range of 0.65 – 0.90 indicating non-Fickian drug release. For commercial tablets n values were 1.15 for J and > 1.15 for others.

The present investigation thus established the usefulness of evaluating the existing commercial SR formulations of DS for their *in vitro* performance and was concluded that the potential sustained and controlled release matrix tablets of DS could be prepared by incorporating polymers like CMC, Carbopol and their combinations in optimised ratio.

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Estimation of Nicotine from Gutkha, a Chewable Tobacco Preparation by a New HPTLC Method

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Accepted 7 March 2001
Revised 5 February 2001
Received 27 September 2000

Nicotine is one of the highly toxic and addictive chemicals, belonging to tobacco alkaloids. In the present work, a HPTLC method was developed for the estimation of nicotine in different brands of *Gutkha* available in local market. Diethyl ether extracts of standard nicotine and the sample solutions were spotted on pre-coated TLC Silica gel G60 F_{254} plated and developed using chloroform:methanol:ammonia (60:5:1 v/v) as mobile phase. Densitometric scanning was performed at 255 nm. The linearity was found to be in the concentration range of 200-1000 ng/spot with correlation coefficient of 0.996. The method was validated for precision, repeatability and accuracy. Twenty different brands of *Gutkha* were analysed for the nicotine content and the results were compared with the nicotine content estimated by an UV-Spectrophotometric method. The content of nicotine in different brands of *Gutkha* was found to be in the range of 0.1 to 0.5% w/w.

Gutkha is a chewable tobacco preparation, containing arecanut, lime, catechu, flavors, permitted spices, saffron and tobacco. Tobacco was formerly used in medicine as sedative, antispasmodic, antiinflammatory, laxative, emetic and abortificient[1]. It's use has been super-

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seded by safer and more efficient remedies and it has a propensity for conferring dependence on its users. Tobacco consists of several toxic substances, the major one of them is nicotine. Nicotine is responsible for the addition potentioal of tobacco[2,3].

Various methods have been reported for the estimation of nicotine from tobacco leaves and its preparations. A review of various analytical methods for the determination of nicotine includes TLC, GC, HPLC and MS has been given by Green *et al*[4]. Other techniques includes spectrophotometry[5,6], potentiometry[7], atomic absorption spectroscopy[8], polarimetry[9] and circular dichroism spectrophotometry[10]. In the present investigation, a HPTLC method was developed for the estimation of nicotine from different brands of *Gutkha*.

From the local market, twenty different brands of Gutkha pouches were purchased. All the reagents used for the analysis are of analytical grade. For the preparation of standard solution, stock solution (1 mg/ml) of nicotine was prepared by transferring standard nicotine in to a 125 ml separating funnel and made alkaline with 0.1 ml of ammonia (25%). It was then extracted with diethyl ether (3 X 25 ml). The extract was transferred to a 100 ml volumetric flask and volume was made up with diethyl ether. The standard solution was prepared by diluting 1 ml of the stock solution to 25 ml with diethyl ether in a volumetric flask.

For sample solution preparation, the contents of one pouch / packet of Gutkha was accurately weighed and transferred to a dry porcelain dish. Two ml of 0.5 M ethanolic potassium hydroxide solution was added to it and triturated well. The mixture was allowed to dry for 30 min at room temperature. The content was then transferred to a 125 ml separating funnel and extracted with diethyl ether (3 X 20 ml). The porcelain dish was washed with 10 ml of diethyl ether twice. The extracts and washings were combined and filtered through Whatman filter paper No. 41 in to a 100 ml volumetric flask and the volume was adjusted with diethyl ether.

Different concentrations (5-25 µl) of the standard solution was applied (5 mn band) on precoated silicagel G 60 F 254 TLC plate (E. Merck) using a CAMAG Linomat IV sample spotter. The plate was developed in a mobile phase comprising of chloroform:methanol:ammonia (6:5:1 v/v) in a glass twin through chamber previously saturated with the solvent system for 30 min. The development distance was 5.5 Cm. After removing from the chamber, the plate was dried, scanned and quantified at 255 nm using a CAMAG TLC scanner 3 and cats 4 software. Data of peak area was recorded. Calibration curve was obtained by plotting peak area vs concentration of nicotine applied.

Sample solution (15 µl) of *Gutkha* was spotted in triplicate along with 15 µl of standard solution of nicotine on a precoated silica gel G60 F_{254} TLC plate (E. Merck) using a CAMAG Linomat IV spotter and analyzed as described under calibration curve. The peak area of sample was recorded and concentration of nicotine was determined using calibration curve for nicotine.

The nicotine content was also estimated from *Gutkha* by a reported UV spectrophotometric method[5] with certain modification in the extraction process of nicotine. In brief, the method is as follows: Sample solution (5 ml) was transferred in to a 100 ml beaker and the solvent was evaporated at room temperature. To this residue, 5 ml of 0.05 N hydrochloric acid was added, mixed well and filtered through Whatman filter paper No. 41. The beaker and the filter paper were washed with 3 ml of 0.05 N hydrochloric acid. The filtrate and the washing solution were transferred to a 10 ml volumetric flask and the volume was made up with 0.05 N hydrochloric acid. The absorbance of the solution was measured at 236 nm, 259 nm and 282 nm using UV - visible spectrophotometer (Shimadzu-180A). From this, $A^{1\%}259$ for nicotine was calculated using the formula

$$A^{1\%}259 = 1.059 \times A_{259} - ((A_{236}+A_{282})/2)$$

The concentration of the nicotine was calculated using Beers-Lamberts equation A = abc, where 'a' is the absorptivity of nicotine from sample at 259 nm.

TABLE 1: SUMMARY OF HPTLC METHOD VALIDATION PARAMETERS

Sl.No.	Parameters	Results
1.	Instrumental Precision (% CV) n=7	0.25
2.	Repeatability (% CV) n=7	2.9
3.	Accuracy (%)	99.29
4.	Specificity	Specific
5.	Linearity Range (ng/spot)	200-1000
6.	Correlation coefficient (r)	0.996
7.	Limit of detection (ng/spot)	50
8.	Limit of quantitation (ng/spot)	200

TABLE 2: RECOVERY STUDY OF NICOTINE BY HPTLC METHOD

Nicotine content in sample (µg)	Nicotine added (µg)	Nicotine found (µg)	% recovery	Average % recovery
89.6	4.45	94.00	99.95	
89.6	8.90	96.48	97.95	99.29
89.6	13.35	102.91	99.96	

Nicotine is of considerable medicinal significance because of it's toxicity and propensity for conferring a dependence on its users. The complex and often unpredictable changes that occur in the body after the administration of nicotine are not only due to it's action on variety of neuro effectors and chemosensitive sites but also to the fact that the alkaloid can stimulate and de sensitize the receptors[2,3]. Moreover, It markedly stimulates the CNS, which is one of the main reason for the consumption of Gutkha. Gutkha may trigger a rare and incurable disease called Submucus Fibrosis (SMF), leading to mouth cancer.

In the present experiment, HPTLC method was developed for the estimation of nicotine. The method was validated for specificity, precision, accuracy and repeatability (Table 1). The method is specific, since it was found that the peak of nicotine was well resolved in the presence of other components of Gutkha. The R_f value for the nicotine was found to be 0.48. The band of nicotine from the sample was confirmed by comparing the UV

TABLE 3: ESTIMATION OF NICOTINE CONTENT IN DIFFERENT BRANDS OF GUTKHA

Sample	Weight of contens per pouch (g)	% w/w of nicotine by	
		HPTLC method	UV method[5]
A	1.88	0.22	0.21
B	1.94	0.18	0.17
C	1.87	0.18	0.17
D	2.00	0.19	0.17
E	1.98	0.18	0.16
F	1.42	0.26	0.25
G	1.97	0.20	0.19
H	1.54	0.42	0.41
I	1.64	0.18	0.18
J	1.30	0.39	0.37
K	1.62	0.30	0.28
L	2.17	0.18	0.16
M	1.98	0.48	0.45
N	1.66	0.31	0.30
O	1.64	0.14	0.12
P	1.50	0.23	0.21
Q	2.13	0.19	0.17
R	1.98	0.17	0.16
S	1.69	0.22	0.21
T	2.06	0.12	0.11

spectrum of the standard band with corresponding sample band using a CAMAG TLC scanner 3. Linear relationship was obtained in the concentration range of 200-1000 ng/spot for nicotine with a correlation coefficient of 0.996. The precision of the instrument was checked by repeated scanning of same spot for seven times and % RSD was found to be 0.254. The repeatability of the method was tested by analyzing 600 ng/spot of the standard solution of nicotine after application on TLC plates (n=7). The result showed a % RSD of 2.9. Accuracy of the method was evaluated by measuring the recovery and average pecentage recovery was found to be 99.29% (Table 2).

The validated method was adopted to dtermine the content of nicotine from different brands of *Gutkha*. The contents of nicotine was found to varying from 0.1 to 0.5% w/w. The results obtained by HPTLC was compared with the results of UV spectrophotometric method. The comparative data showed that there is no significant difference in results of two methods (Table 3).

In conclusion, percentage nicotine content in different of brands of Gutkha was estimated by newly developed HPTLC method. The findings of this study indicates that all the brands of Gutkha containes considerable amount of nicotine (0.1 to 0.5% w/w).

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Antibacterial and Antifungal Activities of Spiroazetidinones

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Accepted 7 March 2001
Revised 12 February 2001
Received 28 April 2000

Spiroazetidinones synthesized from the reaction of diphenylketene, generated *in situ* from thermal decomposition of azibenzil, with 3-arylimino-1-methylindol-2-ones and with 3-aryliminobornan-2-ones have been screened for antibacterial and antifungal activities. The compounds have considerable antibacterial and antifungal activities.

Azetidinones have been studied thoroughly due to their potential biological properties. Several azetidinones with antibacterial, antifungal, herbicidal, antiinflammatory and anticonvulsant activities have been reported[1]. Sulfazecin, isolated from Pseudomonas species and chromobacterium violaceum, are reported to have marked activity against Gram negative bacteria[2]. Therefore, it was

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