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Preparation and Preliminary Stability Study of Immediate Release Theacrine Tablets

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Abstract

Background: Theacrine (1, 3, 7, 9-tetramethyluric acid, TC) is a component of bitter tea from Jinping County, Yunnan, China. It is safe, non-toxic and has many pharmacological effects such as anti-Parkinsonism, reducing anxiety and fatigue and so on. It attracts much attention for further clinical use development. Aim: The purpose of this work was to investigate the stability of TC and its compatibility with commonly used excipients for preparing tablets, thus to develop immediate release TC tablets and investigate the preliminary stability of the tablets. Methods: To figure out the excipients available, compatibility of TC with excipients was measured by storing the samples under 60 °C, relative humidity (RH) 92.5%±5% or 75%±5%, and 4500 Lux ± 500 Lux for 10 days, respectively. The tableting process and formulation was optimized to develop immediate release TC tablets by single factor test. The preliminary stability of TC tablets was investigated by stress testing. Results: Except pregelatinized starch, TC showed good compatibility with other excipients commonly used for tablets. Immediate release TC tablets were developed by direct compression, with croscarmellose sodium (cCMC-Na) as disintegrant, magnesium stearate (MS) as lubricant, mixture of lactose and microcrystalline cellulose (MCC) as filler. The weight variation, friability, disintegration and dissolution of the TC tablets were all qualified. The results demonstrated complete release of TC at 45 min with an accumulative dissolution percentage of 100.07%. The results of stability test showed that the TC tablets were not sensitive to temperature and light, however, moisture absorption of the tablets was observed when stored under high RH of 92.5% and 75%. The weight increased 5.74% and 1.70% when RH was 92.5% and 75%, respectively. Conclusion: Immediate release TC tablets of good quality and stability were successfully developed by direct compression process, and moisture-proof preservation is necessary.

Keywords

Theacrine; Immediate release tablets; Quality examination; Preliminary stability studies.

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1. INTRODUCTION

Theacrine (1, 3, 7, 9-tetramethyluric acid, TC) is safe and has kinds of effects like anti-Parkinsonism, reducing anxiety and fatigue and so on. In this work, immediate release tablets of theacrine were prepared by direct compression process, with croscarmellose sodium as disintegrant, magnesium stearate as lubricant, lactose and microcrystalline cellulose as filler. The tablets showed stable and controllable quality and good stability except moisture absorption under high relative humidity. The weight variation, friability and dissolution of the theacrine tablets were all qualified and the tablets exerted fast and complete drug dissolution. The prepared theacrine tablets were suitable for further development for future clinic use.

Theacrine (1, 3, 7, 9-tetramethyluric acid, TC) is an unusual purine alkaloid. Zheng et al.1 have demonstrated that theacrine is synthesized from adenosine via caffeine in leaves of Camellia assamica var. kucha. Theacrine is synthesized by the metabolism of caffeine through oxidation, isomerization and methylation [1, 2]. The xanthine alkaloids with similar structure, such as caffeine, have strong lipid solubility and are easy to cross the blood-brain barrier [3]. Studies have found that theacrine can also be absorbed into blood and pass across blood-brain barrier after oral administration [4]. So, it is speculated that theacrine also has certain central nervous function [5]. Wang [6] established Parkinson's disease models in vivo and in vitro, and found that theacrine significantly protected dopaminergic neurons by activating Sirt3, thus to increase the activities of related mitochondrial proteins of SOD2, complex II and V to reduce ROS production and increase ROS clearance, which inhibited apoptosis. Moreover, theacrine can reduce anxiety and fatigue [7]. Feduccia et al. [8] observed that theacrine remarkably enhances activity, which is mediated by both adenosinergic and dopaminergic systems. Li et al. [4] found that oral administration of theacrine significantly inverted the learning and memory impairment caused by central fatigue, and restored the levels of fatigue related neurotransmitters in the brain of restraint mice, including 5-hydroxytryptamine, dopamine and their metabolites.

Theacrine is safe and non-toxic. Chinese often drink Kucha containing theacrine, which has been known to have beneficial effects on health [5]. In addition, Taylor et al. [9] gave theacrine to 21 healthy men and women for 8 weeks at 300 mg daily, and the results showed that the values of all fasting clinical safety markers (heart rate, blood pressure, lipid profiles, hematological blood counts, biomarkers of liver/kidney/immune function) were within the normal range. Clewell et al. [10] also concluded from long-term toxicity experiments that the no observed adverse effect level (NOAEL) of theacrine was considered to be 180mg/kg bw/day in Wistar rats. The results showed the good safety of theacrine. To sum up, the various pharmacological effects and the safety of theacrine made it of expectable medicinal application prospect. In this study, we intend to develop a kind of immediate release theacrine tablets for further medicinal development of theacrine. The compatibility of theacrine with excipients, preparing process and formulation screening of theacrine tablets, and the quality examination and preliminary stability of the tablets were studied.

2. MATERIALS AND METHODS

2.1. Materials

Theacrine was synthesized with a purity not less than 98.5%. Microcrystalline cellulose (MCC), magnesium stearate (MS), croscarmellose sodium (cCMC-Na), crospovidone (cPVP), low-substituted hydroxypropyl cellulose (L-HPC), aerosil, talc, pregelatinized starch (PS) were obtained from Shanghai Houcheng Fine Chemical Co. Ltd. (Shanghai, China). Lactose, starch, sodium carboxymethyl starch (CMS-Na), povidone (PVP), carboxymethyl cellulose sodium

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(CMC-Na) were obtained from Shanghai Aladdin Co. Ltd. (Shanghai, China). All other chemicals were commercially available and analytical grade.

2.2. Compatibility Test of Theacrine and Excipients

The pre-experiment found that the tested excipients distinctly absorbed moisture under RH of 92.5%, and the weight gain was 7.5% (>5%). Therefore, the test was performed under RH of 75%. Theacrine was mixed well with the tested excipients at appropriate ratio, and the mixtures were stored under condition of high temperature (60°C), high humidity (RH 75% ± 5%) and stress light (4500 Lux ± 500 Lux) for 10 days, respectively. The appearance, content, moisture absorption was measured to figure out the excipients used for formulation optimization of theacrine tablets. The content was measured using high performance liquid chromatography (HPLC) method developed previously.

2.3. Process and Formulation Optimization of Immediate Release Theacrine Tablets

Direct compression and wet granulation tableting process were used to prepare immediate release theacrine tablets, respectively. The theacrine tablets (10 mg theacrine per tablet) were prepared smoothly by both preparing process, and the tablets prepared by direct compression showed better friability and fast drug dissolution. Thus, the preparing process was determined as direct compression. Briefly, theacrine was simply mixed well with appropriate amounts of excipients, sand the powder mixture was directly compressed into tablets. The sort and amounts of fillers, disintegrants and lubricants was optimized using single factor test, and weight variation, friability and dissolution behavior of the tablets was measured for evaluation. The formulations were listed in Table 1.

Table 1. Formulations of immediate release theacrine tablets.

Component	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16	R17
Theacrine	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Starch	86.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lactose	—	86.7	—	86.7	86.7	86.7	88.7	84.7	86.7	86.7	86.4	86.0	21.7	28.9	43.35	57.8	65.0
MCC	—	—	86.7	—	—	—	—	—	—	—	—	—	65.0	57.8	43.35	28.9	21.7
cCMC-Na	3.0	3.0	3.0	—	—	—	1.0	5.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
L-HPC	—	—	—	3.0	—	—	—	—	—	—	—	—	—	—	—	—	—
cPVP	—	—	—	—	3.0	—	—	—	—	—	—	—	—	—	—	—	—
CMS-Na	—	—	—	—	—	3.0	—	—	—	—	—	—	—	—	—	—	—
MS	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	—	—	0.6	1.0	0.3	0.3	0.3	0.3	0.3
Aerosil	—	—	—	—	—	—	—	—	0.3	—	—	—	—	—	—	—	—
Talc	—	—	—	—	—	—	—	—	—	0.3	—	—	—	—	—	—	—

2.4. Preliminary Stability of Immediate Release Theacrine Tablets

The stress testing was performed to investigate the preliminary stability of immediate release theacrine tablets. Theacrine tablets were store under condition of high temperature (60°C), high humidity (RH 75% ± 5%) and stress light (4500 Lux ± 500 Lux) for 10 days, respectively. The appearance, weight change, content, related substances and dissolution behavior were measured for evaluation.

2.5. Statistical Analysis

The data were expressed as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. Compatibility Test of Theacrine and Excipients

The results of compatibility test were shown in Table 2. Thirteen excipients commonly used for preparing tablets were tested, including lactose, microcrystalline cellulose (MCC), pregelatinized starch (PS), starch, croscarmellose sodium (cCMC-Na), low-substituted hydroxypropyl cellulose (L-HPC), crospovidone (cPVP), sodium carboxymethyl starch (CMS-

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Na), magnesium stearate (MS), aerosil, talc, povidone (PVP), carboxymethyl cellulose sodium (CMC-Na). The appearance of all the tested mixture powders remained off-white powder without change after stored under 60°C, RH (75% ± 5%) and (4500 ± 500) Lux for 10 days, respectively. Except in mixture of theacrine with PS, the content of theacrine in mixture with other excipients was mostly within 90-110% of the initial value after 10-day storage under different stress testing condition, respectively. Also, only the weight gain of mixture of theacrine with PS was higher than 5% after 10-day storage under RH 75%. The results indicated that the other tested excipients were suitable for formulation optimization except PS.

Table 2. Results of compatibility tests of theacrine and different excipients

Excipient	Test item	0 d	RH 75%±5%		60°C		(4500±500) Lux	
			5 d	10 d	5 d	10 d	5 d	10 d
Lactose	Weight gain (%)	—	0.83	1.75	—	—	—	—
	Content (%)	100	99.12	98.57	101.45	102.16	109.13	115.41
MCC	Weight gain (%)	—	2.36	2.80	—	—	—	—
	Content (%)	100	96.48	99.10	105.77	109.06	104.92	109.60
PS	Weight gain (%)	—	4.18	5.58	—	—	—	—
	Content (%)	100	61.59	77.81	60.51	55.80	94.88	90.63
Starch	Weight gain (%)	—	1.99	2.51	—	—	—	—
	Content (%)	100	98.26	96.98	105.27	98.46	100.89	103.06
cCMC-Na	Weight gain (%)	—	2.17	2.31	—	—	—	—
	Content (%)	100	105.32	103.81	104.56	110.75	103.97	103.86
L-HPC	Weight gain (%)	—	0.60	1.25	—	—	—	—
	Content (%)	100	108.25	98.95	102.51	103.25	107.01	99.32
cPVP	Weight gain (%)	—	0.94	1.53	—	—	—	—
	Content (%)	100	106.05	105.94	105.04	108.37	107.84	106.30
CMS-Na	Weight gain (%)	—	0.31	1.22	—	—	—	—
	Content (%)	100	101.46	103.60	106.03	110.95	110.85	104.39
MS	Weight gain (%)	—	1.89	2.22	—	—	—	—
	Content (%)	100	97.12	96.48	100.92	103.54	105.35	103.34
Aerosil	Weight gain (%)	—	0.67	1.03	—	—	—	—
	Content (%)	100	103.26	102.32	101.95	100.66	103.95	101.05
Talc	Weight gain (%)	—	3.52	4.34	—	—	—	—
	Content (%)	100	107.44	106.76	102.30	105.55	100.24	102.61
PVP	Weight gain (%)	—	1.51	2.21	—	—	—	—
	Content (%)	100	102.09	101.84	102.01	100.40	98.19	98.42
CMC-Na	Weight gain (%)	—	3.31	4.27	—	—	—	—
	Content (%)	100	101.09	100.29	102.85	103.63	104.39	102.97

3.2. Process and Formulation Optimization of Immediate Release Theacrine Tablets

In our pretest, theacrine tablets were prepared by both wet granulation tableting process and direct compression. Direct compression was finally selected due to the better quality in friability and drug dissolution of tablets prepared as well as the simplicity of the process.

By using direct compression, small white round immediate release theacrine tablets of 0.1 g were prepared with labeled amount of 10 mg/tablet. The results of weight variation, friability and accumulative dissolution rate expressed as percentage at 10 min (Q10) and 45 min (Q45) of the tablets prepared by different formulations were listed in Table 3. When using starch as filler, the powder mixture showed poor compressibility and compactability, which could not be compressed into tablets. Theacrine tablets of good appearance were smoothly prepared with lactose or MCC as filler, both tablets showed fast drug dissolution. The sort and amount of disintegrants obviously affected the drug dissolution, even resulted in unqualified friability of tablets in certain case. Among the four kinds of disintegrants, the tablets prepared by cCMC-Na, which is characterized by good compressibility and strong disintegrating force [11], showed the

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fastest dissolution, and the optimal amount was 3%. The sort of lubricants showed influence on the drug dissolution to some extent, and the tablets prepared with talc showed the fastest and most complete drug dissolution. The amount of talc demonstrated a little bit influence on the quality of tablets, and the fastest dissolution was achieved at the lowest amount of 0.3%, for which the hydrophobicity of talc partly accounted. Also, the ratio of lactose to MCC in fillers was screened. Although using the mixture of lactose and MCC as filler led just a slight change in drug dissolution of theacrine tablet, the mixture filler (lactose: MCC = 1:3) was chosen due to good compressibility and binding action of MCC in direct compression process. The formulation R13 was determined as the optimal formulation, and the prepared immediate release theacrine tablets were of qualified weight variation and friability, and the drug dissolution rate reached 98.51% at 10 min.

Table 3. Quality of theacrine tablets prepared by different formulations. ($\bar{x}$ ± S, n = 3)

Formulation	Weight variation (%)		Friability	Q10* (%)	Q45* (%)
	Qualified or not	>±7.5(Tablet)			
R2	Qualified	0	<1%	95.10±8.71	97.61±9.61
R3	Qualified	0	<1%	87.13±0.76	87.38±1.44
R4	Qualified	0	<1%	27.33±8.51	75.44±11.38
R5	Qualified	0	<1%	58.33±19.35	94.15±4.94
R6	Qualified	0	<1%	73.01±11.85	91.93±4.84
R7	Qualified	0	>1%	88.18±2.46	83.48±5.99
R8	Qualified	0	<1%	87.02±6.09	90.54±4.06
R9	Qualified	0	<1%	86.89±1.71	83.33±1.99
R10	Qualified	0	<1%	93.97±5.23	94.51±3.55
R11	Qualified	0	<1%	91.17±6.48	91.49±6.92
R12	Qualified	0	<1%	94.50±2.90	91.04±3.27
R13	Qualified	0	<1%	98.51±1.42	100.07±1.17
R14	Qualified	0	<1%	90.16±2.64	91.66±5.21
R15	Qualified	0	<1%	93.89±1.47	94.01±2.73
R16	Qualified	0	<1%	94.04±2.33	94.75±1.26
R17	Qualified	0	<1%	96.30±1.20	99.19±1.89

*Q10 and Q45 are the accumulative dissolution rate of tablets at 10 min and 45 min, respectively. If the weight loss in friability test is less than 1%, the friability of the tablets is qualified.

Three batches of immediate release theacrine tablets of stable quality were prepared using formulation R13 with qualified weight variation and friability. The three batches of theacrine tablets showed uniform content and the content was (9.74 ± 0.02), (9.58 ± 0.20) and (9.99 ± 0.11) mg/tablet, which was within 95-100% of labeled amount. The dissolution curves plotted by accumulative dissolution rate (Q) versus time were illustrated in Figure 1, which showed fast and complete dissolution of theacrine from the tablets.

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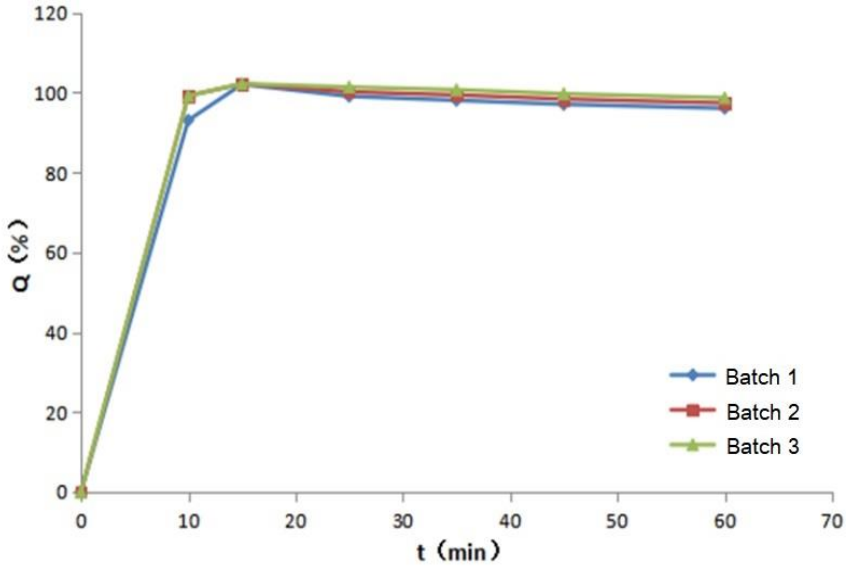

Figure 1. The dissolution curves of three batches of theacrine tablets prepared by optimal formulation R13.

3.3. Preliminary Stability of Immediate Release Theacrine Tablets

Stress testing generally disclosed the preliminary stability of pharmaceutical preparations, and the results provided valuable information for manufacturing environment control, packaging material selection and storage condition determination [12-14]. In the pretest, it was found that the weight gain of tablets was 5.74% when exposed to RH of 92.5%, thus the RH of high humidity was chosen as 75%±5%. The results of stress testing of immediate release theacrine tablets prepared by formulation R13 were listed in Table 4. After 10 days of storage under high temperature (60°C), high humidity (RH 75% ± 5%) and stress light (4500 Lux ± 500 Lux), the white round tablets remained almost no change in appearance and quality, but only a little bit moisture absorption of 1.7% weight gain occurred under exposure to RH 75% ± 5% for 10 days. No impurity was observed in HPLC chromatograms after 10 days of exposure to the extreme test conditions, and the content of theacrine kept stable within 9.91 ± 0.26 to 10.42 ± 0.33 mg/tablet, which was 99.1% to 104.2% of the labeled amount. The dissolution of the tablets also demonstrated no distinct change, and the dissolution curve after 10 days of test almost coincided with that before test (Figure 2). It followed that the developed immediate release theacrine tablets were stable under the test condition.

Table 4. Results of stress testing of immediate release theacrine tablets exposed to 60℃, RH 75% ± 5% and (4500 ± 500) Lux. ($\bar{x}$ ± S, n = 3)

Exposed condition	Days	Weight change (%)	Content (mg/tablet)
60℃	0	0	10.11 ± 0.48
	5	-0.01	10.15 ± 0.27
	10	-0.01	10.12 ± 0.45
RH 75% ± 5%	5	1.61	10.04 ± 0.52
	10	1.70	9.91 ± 0.26
(4500 ± 500) Lux	5	0.01	10.17 ± 0.34
	10	0.00	10.42 ± 0.33

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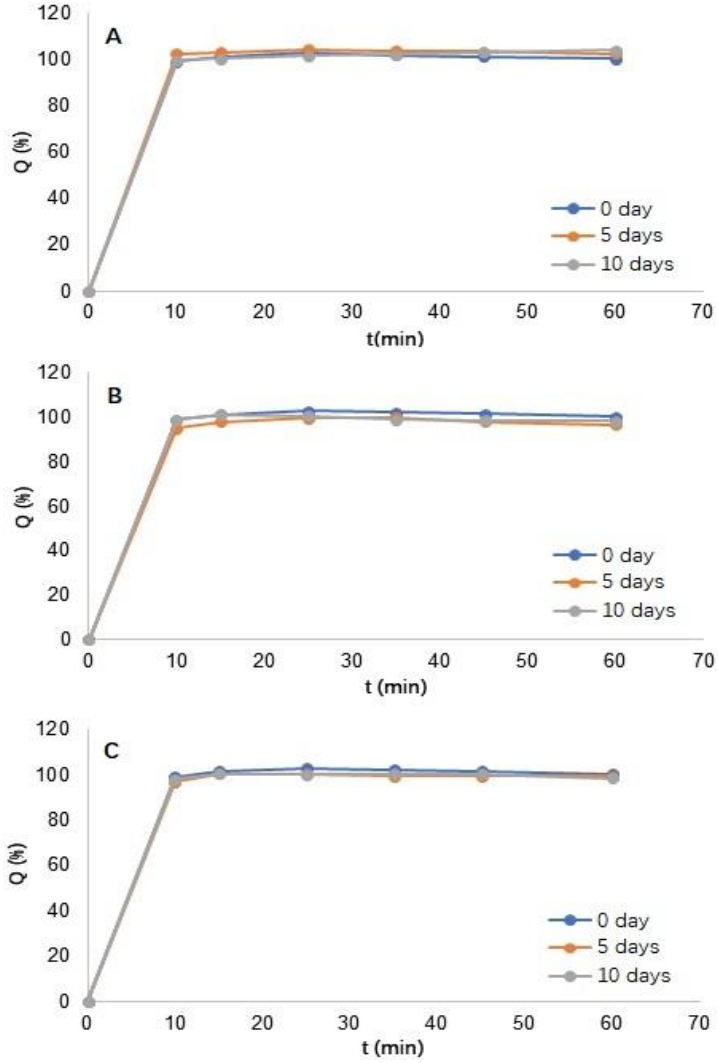

Figure 2. Dissolution curves of theacrine tablets exposed to 60°C (A), RH of 75% ± 5% (B) and (4500 ± 500) Lux (C).

4. CONCLUSION

In conclusion, immediate release theacrine tablets were prepared by direct compression with mixture of lactose and MCC (1:3) as filler, cCMC-Na as disintegrant and talc as lubricant. The tablets had stable controllable quality with good repeatability, and the drug dissolution was fast and complete. The results of stress testing indicated theacrine tablets were of good stability under the extreme test conditions, only a little bit high weight gain was observed under RH of 92.5%, thus damp proof packaging was preferred.

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Authors contribution: Sha Li conceived the idea, designed and supervised the research. Yulin Zhu and Junming Lin performed the research, conducted the analysis and wrote the paper. Wen Huang and Guizhi Weng contributed to data collection and analysis. All authors contributed to the writing and revision.

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